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*Because of the space necessary and the fact that only a minority would be interested in the individual case histories we have not deemed it advisable to publish them with the present report. They will be published with the reprint of this article in the Collected Studies from the Research Laboratories, copies of which may be had by application to the Librarian.

INTRODUCTION.

The idea that part of the tuberculous disease in man was to be traced to infection from cattle, had its beginning in the early part of the nineteenth century. Specific attempt at proof was first made by Klenke in 1846, who insisted on the infectiousness of milk from tuberculous cattle. The results of the inoculation experiments culminating in the positive demonstration by Villemin in 1865 of the infectious nature of tuberculous material, from both man and cattle, for other animals strengthened this view.

With the discovery of the tubercle bacillus by Koch in 1882, the unity of tuberculous disease in man and the lower animals was apparently settled. Although differences in virulence were noticed in material from man and cattle, and had been noticed even before the discovery of the tubercle bacillus, they raised no doubt as to the complete identity and intercommunicability of the disease. The first step towards separation of varieties among tubercle bacilli was taken when Rivolta and Maffucci in 1889–1892 showed that the avian tubercle bacillus differed in many ways from the mammalian type. In 1896 Theobald Smith* published a study of two cultures, one from a pet animal (*Nasua narica*) presumably infected by its tuberculous owner; the other from a tuberculous bull. As a result of the differences noted he concluded that more evidence as to the identity of bacilli from bovine and human sources was needed. It is an interesting fact that on the examination of these two cultures alone he determined every essential difference between the two types. He published further evidence in 1898 of the existence of a human and bovine type of bacillus.

In looking backward over the development of this knowledge as to the existence of varieties, it is peculiar that Koch should not have noticed these differences in his original work. This, however, is probably due to the fact that in the endeavor to prove the causal relationship of the tubercle bacillus to

* To avoid continual cross-references in the text, the literature cited will be given at the end, arranged alphabetically according to author. The title will be given to render selection easier to those wishing to use the references.

tuberculosis, quantitative differences were lost sight of. The careful comparative work of Vagedes in 1898, which was done under his direction, seems to have raised no doubts as to the essential identity of the bacillus from man and cattle; though the possibility of different types was in his mind and probably the incentive of this investigation.

In the face of the growing evidence of varietal differences, little doubt was entertained as to the infectiousness of the bovine virus for man. Much work was being done during this time on the infectiousness of milk from tuberculous cows, and the proportion of tuberculosis in man which could be considered of intestinal origin. Further, many cases were reported as infections from tuberculous cattle, on the evidence furnished by the probable source of infection and the type of disease. The cases of accidental inoculations with the bovine virus in post-mortem work and under other conditions were considered final evidence.* The simple procedure of investigating the type of bacillus isolated from different forms of tuberculosis, and by observation of the cultural and virulence characteristics, in comparison with known cultures, to determine the source of infection, as advised by Smith, received little attention. Interest in the question suddenly became acute with the sensational announcement of Koch in 1901.

The prominence of the man and the startling character of his conclusions, which were practically a reversal of his original views, aroused interest in the subject. The statements he made were as follows:

"In view of all these facts I believe that I am justified in maintaining that human and bovine tuberculosis are not identical, and that the human variety cannot be transferred to cattle. . . . But how about the susceptibility of human beings to bovine tuberculosis? This question cannot be answered directly since, of course, experiments on human beings are not permissible; but we can get at a solution

*For complete summaries see Salmon, Relation of Bovine Tuberculosis to the Public Health, Bureau of Animal Industry, Bulletin No. 33, 1901; and Kober, The Transmission of Bovine Tuberculosis by Milk, with a tabulation of eighty-six cases, Trans. of the Assoc. of Am. Phys., Vol. 18, 1903.

indirectly. It is a well known fact that much of the butter and milk consumed in large cities contains a considerable number of living tubercle bacilli (Perlsucht-bacilli). If these are pathogenic to human beings we ought to find among the inhabitants of large cities, who daily swallow these living virulent organisms, and thus unconsciously carry out the very experiment which we cannot undertake, many cases of tuberculosis that can be traced to food contaminated with the tubercle bacillus; especially ought this to be true in children. . . . In reality it is not so. We can admit infection with tuberculosis through food containing tubercle bacilli only when the intestine is the seat of the first lesion, *i.e.*, in cases of primary intestinal tuberculosis. Primary intestinal tuberculosis, however, is extremely rare. . . . And, in the few cases reported, it has not been conclusively proven that we were dealing with an infection with bovine tuberculosis. The chances are equally in favor of its being of the human form. . . .

"To-day we are able to make a diagnosis. It is necessary merely to isolate the organism from the tuberculous material in pure culture, and by inoculation into cattle establish whether it is of the human or the bovine variety. . . .

"For half a year I have experimented along these lines but, owing to the scarcity of material, have been able to examine only a few cases. So far, results do not indicate the occurrence of bovine tuberculosis in man.

"Although the question whether man is susceptible to bovine tuberculosis has not been definitely answered, and probably cannot be immediately answered, we may safely say that if such susceptibility does exist the infection takes place rarely. I should be inclined to consider the number of cases of tuberculosis caused by milk, butter or meat of tuberculous cattle not much greater than that of hereditary cases, and I believe, therefore, that no special protective measures are indicated."

These views found violent opposition in most quarters. Numerous investigations followed as to the identity or non-identity of the bacillus from man and cattle. At the same

time the viruses from man were investigated as to whether they were identical with those from cattle or not. The resulting evidence left no doubt of the infectiousness of the bovine virus for children, but in the case of adults this was not established.

The importance of the question from the human standpoint rests on the percentage of such infection. The control of tuberculosis in cattle is probably necessary anyway, from a purely commercial standpoint. If, however, bovine tuberculosis is a menace, that is, causes an appreciable percentage of the tuberculosis in man, the control involves the production of milk and its products free from any possible infectious material. With a milk supply such as that of New York and other cities, which, of average quality, yields tubercle bacilli in six to sixteen per cent of the samples examined, the changes necessary to render this supply innocuous are far reaching. The real solution of such a sanitary problem must rest on the incidence of bovine infection in man deduced from the examination of a large series of *non-selected* cases of different types of disease. Further, these cases must be considered from the standpoint of disablement or death caused by bovine infection.

The numerous investigations, especially those of American workers, and of the English and German Commissions, have settled many of the disputed points. Nearly all the cases examined, however, have been selected either with the view of finding bovine infections, that is, tuberculosis of alimentary origin, or adult pulmonary tuberculosis. It seems to us with the knowledge at hand that a large series of non-selected cases of every type of disease obtainable and, further, cases under average conditions of exposure to infection, would add to our knowledge and give information of great importance in sanitary control. Further, any additional technical points, especially tending to the very simplest methods of differentiation, would be of value. In the examination of such a large series of cultures we hoped that many disputed or doubtful points in biology or virulence could be settled.

METHODS OF ISOLATION, CULTIVATION, AND MEDIA EMPLOYED.

Isolation. — No direct cultures were attempted. The material sent to the laboratory was, with the exception of operative material, all secondarily contaminated. For methods of comparison we preferred to have all the cultures isolated in one way, so that the guinea-pig * has been used in every case. The material emulsified in salt solution was injected into the thigh, subcutaneously or intramuscularly, and the animal killed about three to five weeks later without violence, either with chloroform or illuminating gas. If the pig should die, cultures may be attempted, the shorter the time after death the better the chances of avoiding contamination. We have simply stretched the pigs in trays and, after thoroughly soaking the hide with five per cent carbolic acid, removed the tuberculous lymph nodes and spleen to separate Petri dishes. The tissue was then thoroughly minced with knife and forceps, and by means of a stiff platinum wire thoroughly smeared over the surface of the culture media used. The number of tubes to be made depends on one's skill in avoiding contamination. The method advised by Smith of simply placing the cut tissue on the surface of the media and at the end of a week to ten days rubbing it over the surface to spread the bacilli which have already grown seems to us unnecessary. The second opening of the tube, a fruitful source of contamination with moulds, is avoided by our method, and the growth is as good and even greater in amount. In a very few instances, however, with bovine viruses where growth was apparently absent at the end of three weeks, we have opened the tubes and again rubbed the tissue over the surface of the media and on further incubation growth became visible. Whether this was due to the spreading of a very few fine colonies, which were due to the presence of very few bacilli in the tissue inoculated, or whether the added incubation allowed the further growth of

* There is no evidence whatever that direct cultures differ in any particular from cultures through the guinea-pig. The pig, however, gives a greater abundance of bacilli, and cultures tend therefore to be more uniform and allow earlier deductions.

a very reluctantly developing virus, we are not able to say. Probably both factors were at work. Under these conditions, which seldom arise, the method seems of value.

Certain points of practical importance have been noted during the course of the work. The material best suited for cultures seems to be the lymph nodes, either the inguinal or a large retroperitoneal node at the upper lumbar region. The spleen is not quite as favorable. Occasionally a suppurative adenitis of the inguinal nodes interferes with their use. The lungs have given a large proportion of contaminated tubes and their use was discontinued. As to the size of the guinea-pig, small pigs seem better, that is, they are less resistant and the greater degree of tuberculosis gives more tissue for use. Occasionally the presence of tuberculosis is doubtful on macroscopic examination of the pig. In this case, besides injecting the suspicious organs into a second set of pigs, we have made cultures and in some instances with success. This attempt, though it may fail, is worth while, in that time is saved if successful. The injection of a second series of pigs is sometimes necessary to obtain sufficient material for use, probably because of the very few bacilli in the original material.

In all cases egg media have been used for isolation. The ease with which this medium can be obtained and the uniformity of results make other media unnecessary. The tubes were observed at the end of one week to avoid the possibility of error due to some rapidly growing acid fast organism. We have had no trouble in this respect, except that in a few instances pigs injected with sputum have given cultures of streptothrix-like organisms. A second series of pigs injected from the first set in this case has weeded out this contamination and given pure cultures of tubercle bacilli.*

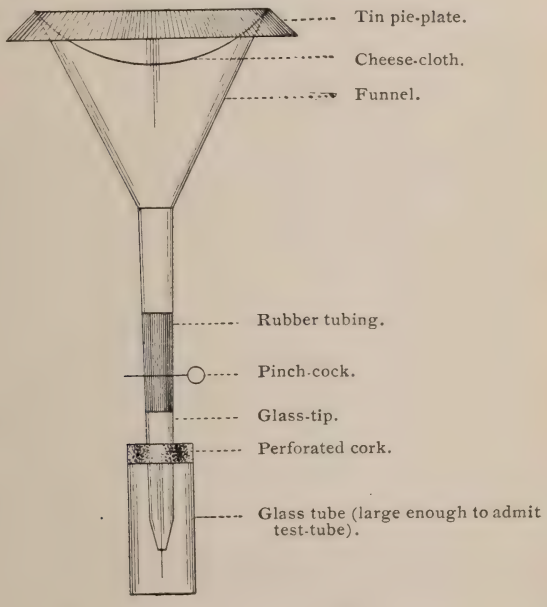
Culture media. — Various media have been used both for isolation and further cultivation. The following is a description of the methods employed:

* This seems to us to throw some doubt on the reports of streptothrix infections. The possibility that this was only a secondary invader according to the above experience would not be excluded.

Egg media of two types have been used. The eggs were secured as fresh as possible.

Plain egg (Dorset). — The eggs are thoroughly cleaned with water of any adherent dirt and then washed with five per cent carbolic solution and allowed to partially dry. The ends of the eggs are then gently dried in the flame and pierced with a burned sharp forceps. The hole at one end should be about three-eighths of an inch in diameter, and the membrane broken; the other end which is to be blown into should be smaller and the membrane left unbroken if possible. The eggs are then blown into a sterile Erlenmeyer flask, the blowing being done from the cheeks, which

Straining and Filling Outfit.



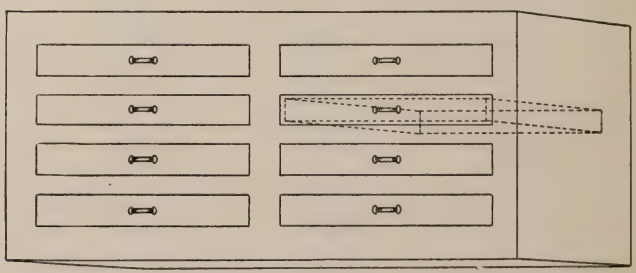
The lower tube is plugged with cotton and the top wrapped. It is necessary to loosen the pinch-cock before heating. The whole is sterilized in the autoclave.

will help to avoid spilling saliva and leakage of air around the outside of the egg. To the egg is then added ten per cent of water by volume of the weight of the eggs. The

mixing is done by a twirling motion of the flask or by gently stirring with a glass rod. Bubbling is to be sedulously avoided. The mixture is then strained through cheese-cloth by gravity, and tubed. The accompanying drawing shows a simple home-made outfit copied from the Maassen apparatus, for the last two steps. The contents of the flask are poured on the cheese-cloth and, after straining into the funnel, tubing is done from the dust-protected tip. The tubes are then inspissated at 70° C. for two to two and a quarter hours in a moist chamber. This is all the heating we have employed. If needed, moisture is provided by adding two or three drops of water to each tube, using the same apparatus as above. The cotton plug is cut off and the tube sealed with a tight-fitting cork charred just as it is inserted. With the most rigid precautions as to asepsis and incubation for one week before using, to weed out contaminations, little trouble is encountered even with this short heating.

For inspissation we have devised a water bath which has given the greatest satisfaction, the advantages being that it is always ready for use and that each tube is heated to the same temperature. The accompanying drawing shows

INSPISSATOR.



the details of its construction, the essential point being the compartments open in the front, and projecting backward into the bath with a downward tilt to give the right inclination to the tube of medium to be solidified. Each compartment holds only one layer of tubes. To add moisture a rope of saturated cotton is laid in the side of each compartment.

The openings are closed with a tightly fitting cover, made like the cover of a tin can. The bath is supported by two ring stands and the backward or forward tilt modified to suit the incline of the media in the tubes.

Glycerine egg (Lubenau). — Ten eggs are blown into a flask and two hundred cubic centimeters of glycerine bouillon (five per cent glycerine, neutral or moderately alkaline to litmus) added. The further preparation is as with plain egg, except that further addition for moisture is usually unnecessary. The diluent in this case averages about thirty-five per cent of the weight of the eggs.

To the two kinds of egg media used we have added glycerine bouillon, so that about one-third of the surface of the egg was covered. This makes a convenient medium for pellicle formation, the growth spreading from the egg to the bouillon.

Glycerine potato. — We have followed the method given by the Royal Commission with very good results. Potatoes are cut in the usual Bolton method, soaked in 1–1000 sodium carbonate solution in distilled water for twenty-four hours, drained, and then soaked in five per cent glycerine in distilled water for the same length of time. The potatoes are then tubed, using ordinary three-quarter-inch tubes, and the glycerine water in which they were soaked added, covering about one-third of the surface. Intermittent sterilization in the Arnold sterilizer was employed. In some instances we have for special purposes used glycerine bouillon in place of the glycerine water, as when a large number of pellicles were wanted. The pellicle formation is, however, very good with the ordinary method.

Glycerine agar and bouillon. — These were made in the ordinary method. Veal, beef, and occasionally beef extract were employed.

Glycerine litmus milk. — This media was advised by the Royal Commission, as a substitute for bouillon in eliciting the Smith reaction. Milk is freed from the cream and five per cent glycerine added. The addition of five per cent of a

five per cent watery solution of Merck's purified litmus gives a good color.

Serum media were tried in the early part of the work. Será of the dog and calf were used but most of the batches made were unfavorable. The difficulty of getting dog serum, the efficiency and reliability of the great majority of batches of egg media, caused us to abandon the use of serum almost immediately. It was noted that the same serum which was poor when coagulated gave much better results mixed with agar.

For isolation we have used egg media as already stated. Both types of egg media have been used for isolation in each case. The reasons for using both will be discussed later, it being sufficient to state at this point that glycerine-containing media are usually unfavorable for the isolation of the bovine type of bacillus. The other media have been tried for isolation simply for interest, and though frequently successful they are not so well suited for such work. The value of the media for identification of the type and the degree of difference shown will be given under the cultural characteristics of the two types.

All tubed media after inoculation have been incubated in the inclined position. Though this is not absolutely necessary, we started with this method on the suggestions of previous workers, and continued it purely from the standpoint of uniformity. All tubes have been sealed either with cork alone or, what seems preferable, to dip the cotton stopper also in paraffin. Though the cork alone will conserve the moisture, the paraffin seemed to help exclude moulds. As to moisture, the best procedure is to conserve the moisture in the individual tube, but to keep the surrounding atmosphere reasonably dry. In this way moulds are few and only troublesome in the summer months. We tried keeping the tubes in a closed moist cabinet in the incubator with the result of moulds over everything, and inoculations from these tubes were surely mouldy even though the tubes were wiped clean before transferring.

Frequency of transfer is essential where tubes are being

used for comparison. In this way only can the maximum vigor of growth be continued. Transfers made every three to four weeks are best for this purpose. After six to eight weeks subcultures begin to lose in vigor and luxuriance of growth. For pellicle formation fresh subcultures are very essential.

CULTURAL CHARACTERS OF THE TWO TYPES.

Resumé. — The first attempt to use the cultural differences of the two types as a means of differentiation was made by Smith in 1896.*

He found that on serum the human type grew freely, the growth being difficult to break up, whereas the bovine type grew sparsely and the growth, on lifting with the platinum loop, had little cohesion and was easily broken up. These differences were found to be constant † in the later cultures he isolated.

Ravenel also noticed this difference in amount of growth, and further says that in very early subcultures on glycerine agar the human grew luxuriantly at once, while the bovine grew not at all.

Dorset found that on the egg medium devised by himself two groups could be made on cultural characteristics.

Group 1 (bovine type) showed scanty growth, and in cultures from the guinea-pig the colonies were small, flat and tended to coalesce. The growth in fresh cultures was moist and glistening and could be easily brought into a state of fine subdivision in bouillon. Group 2 (human type) in original cultures showed round elevated colonies and in subcultures the growth was more abundant than in Group 1, and could not be so easily broken up, being also more adherent to the surface of the culture medium.

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* These resumés are in no way complete. The chief historical points and the conclusions of the early workers are mainly noted. Besides this the opinions of those examining larger series of cases are given and of those dissenting from the general conclusions. Other important points will be taken up in connection with the discussion of the technical part of the work.

† Except Sputum I.; this culture was so different from what we should expect of a typical tubercle culture, that it is probable that it was some other organism.

The sharpest separation into two types, depending on the amount of growth, was made by Kossel, Weber and Heuss and by Oehlecker. They used glycerine bouillon as a differential medium. Although differences were noted on plain serum, they were much more marked on glycerine bouillon. The human type usually grew well, covering the surface completely in two to three weeks, climbing on the glass, and forming a uniform thick wrinkled membrane. With the bovine type the growth was much slower and sparser, and unreliable. It consisted of a fine net-like or veil-like pellicle and in many cases this was the extent of the growth. In other cases wart-like thickenings occurred of varying diameter, others grew as a uniform spreading pellicle. These differences are present only in freshly isolated cultures.

Wolbach and Ernst, Moeller, Beck and other investigators have noted the quantitative differences of growth on various media.

The conclusions as to cultural characteristics as given by the Royal Commission are somewhat complicated by the varying results obtained by the different investigators. Furthermore, no absolute routine method was employed, so that comparison of cultures was not always made under identical conditions.

In general, basing their conclusions on one factor, viz., amount and rapidity of growth, they conclude that all cultures fall into two classes — a dysgonic or poor growing class and an eugonic or good growing class. In the first class they place the bacillus of bovine tuberculosis and the bacillus of Group 1 (bovine type) of human tuberculosis. Of Group 1 they say: "The cultural characters of the bacillus of this group are in every respect identical with those of the bacillus of bovine tuberculosis. In all cases the bacillus may be described as dysgonic, though to a varying degree in the several cases, and the range of variation coincides almost exactly with the range of variation shown by the bacillus of bovine tuberculosis." In the second class only cultures of human origin are included (human type). They find, however, that the range of variation in these two classes is so

wide that the better growing cultures of the first class differ slightly if at all from the poorer cultures of the second class, the final result is, therefore, practically an unbroken series.*

The preceding short sketch of some of the work already done shows clearly that the most constant difference noted was in the amount of growth of the two types. The only difference of opinion has been as to the value of cultures as a differential means, and the best method of eliciting these differences. It is conceded by all that these differences tend to disappear in older cultures.

A number of investigators have failed to obtain these sharp differences. The most important dissenting opinions being those of Rabinowitch and Fibiger and Jensen. Though they have found quantitative differences in many of their cultures, they have had so many irregular and exceptional results, in which cultures from human and bovine sources have approached each other, as to question the value of this evidence as sufficient means of differentiation.

Own observations. — The results of our work have led to the conclusion that there is no constant qualitative cultural difference between the human and the bovine types of tubercle bacilli. Quantitatively and with respect to the effect of glycerine, however, there is a marked difference in the great majority of cultures, so great in fact that almost without exception the type can be determined from cultures alone. This difference is constant in one factor only, viz., amount and rapidity of growth in early cultures. In classifying our cultures according to this characteristic we can broadly say that all bovine types of bacilli are dysgonic † (sparse growth) and all human types of bacilli are eugonic (moderate or luxuriant growth). The question remains what is the best method for eliciting this difference. Any medium used must fulfil certain conditions: first, it must be especially adapted for the growth of the eugonic viruses so that the best possible growth is obtained; second, it would

* For a more detailed discussion of their results see page 38.

† Terms used by the Royal Commission to describe the amount of growth.

be preferable if the growth of the dysgonic virus were somewhat retarded on the medium. This will widen the gap as far as possible. Third, the medium must be nearly uniform in its results, that is, growth should not fluctuate with the different batches of media used. We have found that glycerine egg is by far the best medium for this purpose.

Moeller and later Beck had noticed that the addition of glycerine had, as a rule, a restraining action on the growth of fresh bovine cultures. Other authors have denied this. Cobbett (Royal Commission) made comparisons of the two types of organism on both serum and glycerine serum. While the human cultures were far more vigorous with the use of glycerine serum, the bovine cultures were either restrained or, if aided by its presence, the increase in growth was slight. This difference he found in early cultures to be of diagnostic value in the separation of the two types. We also noticed that primary cultures of the bovine type repeatedly failed on glycerine egg, whereas the primary cultures of the human type were usually markedly increased in luxuriance. The reading of Cobbett's results led us to adopt glycerine egg as the basic differential medium.

It may be objected that the presence of bouillon instead of water is the cause of this difference. The bouillon does seem to exert some influence but, as far as controls with differently-made egg media go, the essential factor is the glycerine. The increased amount of fluid, that is thirty-five per cent of bouillon in place of ten per cent of water, is also to be considered. The bouillon and increased fluid seems to stimulate pigment production but influences the growth to a very slight degree.

Giving the results, then, in terms of glycerine, the following general conclusions may be drawn:

a. All cultures growing luxuriantly on glycerine egg from the start are of the human type.

b. All cultures growing sparsely, or even not at all, on glycerine egg in the first few generations are of the bovine type.

Characteristics of the two types on glycerine egg. — Bovine type. — As pointed out by Griffith (Royal Commission) bovine cultures fall into groups according to their growth on glycerine serum as compared with the growth on plain serum. This difference is most marked in primary cultures. Tabulating the primary cultures in cases where comparisons were made the results are as follows:

PRIMARY CULTURES. BOVINE TYPE.

Source of Culture.	Growth Negative or much Poorer on Glycerine Egg.	Growth Equal or nearly so on Glycerine Egg.	Growth Better on Glycerine Egg.
Man	23 or 79% +	5 * or 17% +	1 or 3% +
Cattle (3 milk)	23 or 85% +	3 or 11% +	1 or 3% +
Both combined	46 or 82% +	8 or 14% +	.2 or 3% +

* A duplicate culture from one of these cases was poorer on glycerine egg. In all the other cases the different strains isolated were uniform in this characteristic.

If this table had been made from the second or third generation, even though this was the first generation on glycerine egg, the tendency would be for a shifting of many cultures to the second or even third subdivisions. After some generations on glycerine egg the tendency would be for more and more to fall into the third group. We have not carried out this comparison of the rate of increase of growth on egg, with and without glycerine, completely. As far as we have gone the tendency is for the growth on glycerine egg to outstrip that on plain egg as soon as it has become accustomed to glycerine.

This grouping has indirect bearing on the differentiation of type, showing the gradations or variations of one characteristic, namely, the growth in the presence of glycerine, which is of great importance in the differentiation of the two types.

The character of the growth itself on glycerine egg varies with the vigor of the cultures. The poorest cultures may show only a few small isolated colonies, while the better

growths form a complete layer. One of the most noteworthy features of the growth on this medium is the tendency to moistness. This will vary so that the culture may have a slightly moist granular appearance or, in the other extreme, be almost wet, showing the course of the needle in transferring the growth, giving it a streaked, at times, almost slimy appearance. To this characteristic is due the fact noted by Theobald Smith, that bovine cultures could be easily subdivided in broth or saline, and a very even suspension obtained. This is very marked in making smears, an even distribution of the bacilli on the slide being comparatively easy. Pigment production is almost uniformly absent in early cultures. When the growth becomes more vigorous on further cultivation pigmentation appears, long incubation aiding its development. The pigmentation varies from pinkish tinge to definite pinkish discoloration of the growth.

After bovine cultures become accustomed to glycerine egg the increase in amount of growth on further cultivation varies with different cultures; some remain persistently sparse for many generations, others increase very rapidly in amount in a few generations. The rapidity of this increase does not seem to be correlated with its reaction to glycerine in the primary culture. After cultivation, however, all viruses tend to change from dysgonic to eugonic. It is absolutely essential, therefore, that all comparative observations be made in the first three or four generations if the degree of dysgony is to be correctly estimated.

Human type. — The bacillus of the human type usually grows luxuriantly on glycerine egg from the start. The increased amount of growth, in the presence of glycerine, is evident in nearly all primary cultures. Where the inoculated material has contained few bacilli this is not so marked, but even here it is noticeable. For the diagnosis of type this difference need not be noted, the growth being usually so luxuriant on glycerine egg that no doubt is entertained as to the type of bacillus.

The character of the growth varies as to whether the colonies are discrete or whether there is a confluent complete

layer of growth. In the former, which only occurs in primary cultures, apparently due to the small number of bacilli in the material inoculated, the individual colonies are large, raised, and rounded. In the latter case the growth is raised, crumpled or coarsely verrucose. Pigment production is marked, especially on transfers, varying from a pinkish tint to very distinct pink or even coppery red. Pigmentation is noticed in primary cultures if incubation be prolonged. A very peculiar feature of this pigmentation is that tubes stored in a cool closet become more deeply pigmented. This has been repeatedly noticed and seems in absolute contradiction to the fact that growth could not take place at this temperature. The growth is dry, and correlated with this is the difficulty of subdividing the growth to form a suspension or smear. It is also tenaciously adherent to the egg, so that the surface is frequently torn in attempting to raise some on a platinum loop. Very exceptionally cultures show slight moistness.

Besides glycerine egg we have used glycerine potato as a routine medium in classifying our cultures. This was for the purpose of having a medium which, containing no coagulable albumins, would give a further index of the saprophytism of the two types. We selected potato as this has been far more uniform than glycerine agar or glycerine broth.

Here again we encountered only one distinctive differential characteristic, viz., amount and rapidity of growth. The bovine type commonly fails to grow in the first few generations. If it does show growth the amount is slight, usually consisting of scattered granules which may be close together forming patches. A pellicle often spreads over the water in the tube. This we have observed as early as the second generation, but the pellicle is exceedingly thin and delicate. With further cultivation the growth increases with varying rapidity, so that it finally becomes equal in amount to the easily growing human types.

The human type of bacillus usually grows very vigorously from the start and nearly every case shows the maximum

vigor of growth in the first few generations. A few exceptional cultures have shown a great deal of reluctance on potato even though the growth has been good on glycerine egg from the start. The growth usually consists of a complete layer of raised granular appearance, and where the water in the tube is sufficient a vigorous, moderate or thick wrinkled pellicle is formed. Pigmentation is common, but the majority of cultures are only brownish in color.

The pigmentation of tubercle cultures on potato has received a good deal of attention due to the statement of Arpad that the formation of an orange to brick red pigment was a distinctive characteristic of the human type. Later investigators have found this very irregular in its appearance, and little weight has therefore been put upon it. If we analyze the results of twenty-five consecutive cultures of the human type the following results give the frequency of appearance of pigmentation. Sixteen viruses showed pigmentation in some generations, nine did not. The total number of transfers on potato was two hundred and one, of which only thirty-one transfers showed pigmentation. That is, only sixty-four per cent of these viruses showed pigmentation and of the total transfers only fifteen per cent. Assuming that this pigmentation was diagnostic, the difficulty of controlling its production makes it of no value. The earliness or lateness of its appearance fluctuates widely. Whether this pigmentation will appear in bovine cultures we cannot say at present. We have, however, in our best growing bovine cultures noticed a suggestive pinkish tinge in some potato tubes. From analogy with the glycerine egg cultures, where pigmentation is common to both and appearing in the bovine cultures with increased vigor of growth, we consider the difference non-specific. It is probably simply a quantitative difference, the faculty being more highly developed by the human type. It would seem to be of diagnostic value only in so far that its appearance in the first few generations is further evidence that one is dealing with a human virus.

The preceding gives the broad general cultural differences

between the two types on glycerine egg and glycerine potato drawn from the characteristics shown by the great majority of cultures. What follows will show the individual variations in these two groups and possible exceptions.

The two media given have formed the basis of our classification of cultures. In a few cases examined before this routine was established, the growth on glycerine agar or broth is used in place of potato, and the classification made as closely as the data permit. It is important that observations be made at a period in cultivation when in the great majority of cases the better growing cultures shall have reached their maximum. Three weeks was found to be a good period, as the growth beyond this time of the eugonic viruses was not marked, and at the same time the dysgonic viruses growing very slowly made the gap very wide. Most of the observations were made at this time. As the number of cultures increased very greatly, the time was gradually lengthened and comparisons have shown that three weeks to one month give equally good and practically identical results. Beyond this the bovine cultures slowly increase, and although the differences in the early generations are very marked we prefer a time limit which gives the maximum differences.

For ease of recording cultural luxuriances numerical grades of growth were adopted. Nine grades were arbitrarily selected, thus Grades 1 to 3, sparse; Grades 4 to 6, moderate, and Grades 7 to 9, vigorous. The total amount of growth was judged by inspection of the cultures. As minor variations occur at times in the individual tubes of a set of transfers, due to accidental conditions, it was the rule in every case to select the best growing tube for recording the grade. It is only reasonable to consider that the best tube is an index of the capacity of growth under the most favorable conditions. It is of the greatest importance that all conditions be uniform and not least of all the skill of the individual worker. Recently the transplanting of the cultures has passed to new hands, and although these hands are trained in the work there are a great number of sudden

irregularities in the cultures; the greatest irregularity being typical good growing tubes and very poor growing tubes in the transfers of the same culture on the same batch of glycerine egg. These irregularities have also been noted where the work of isolation has changed hands. It is too often forgotten that the cultivation of the tubercle bacillus, as some German authors insist, is a *Kunst*. All factors need the most careful control and supervision, if accidental variations are to be avoided. The great majority of the transfers fortunately have been done by one person, and the same has been the case with the observations.

On the basis of the grade of growth on glycerine egg and glycerine potato the following classification has been made. It is to be noted that in this classification whenever the first three generations are spoken of it refers to the total number of generations regardless of the media. In some instances, especially in the bovine cultures, plain egg has been used to start the cultures, and this is included in the generations given. Where, however, five generations on glycerine egg are mentioned it means five successive transfers on this media. If any generations preceded this they are not counted, as they were on glycerine-free media. Grade 9 is not mentioned in the following, as we used it only in a minority of exceptionally luxuriant transfers. Grade 8 was the average maximum luxuriance.

Human type. — Group 1. — Glycerine egg,* Grades 7–8, in the first three generations, showing pigmentation in the early generations. Glycerine potato, Grades 6–8, in the first four generations.

Group 2. — Glycerine egg, same as in Group 1. Glycerine potato (*a*), less than Grade 6, or (*b*), negative or slight, in the first four generations.

Group 3. — Glycerine egg, Grades 4–6, in the first three generations with pigment slight or absent in the early

* The group and type of each virus and its representative strains will be given in the final tabulation of the individual cases with their histories, to be published in the Collected Studies.

generations. Glycerine potato, Grades 3–5, in the first four generations.

Group 4. — Glycerine egg, as in Group 3. Glycerine potato negative or practically so in the first four generations.

Groups 3 and 4 may or may not show increase of growth in the first five generations on glycerine egg or other glycerine media.

Bovine type. — Group 1. — Glycerine egg, Grades 0–3, in the first three generations and growth remaining sparse, or at most increasing slowly in the first five generations on glycerine egg. Glycerine potato, slight or negative in the first four generations.

Group 2. — Glycerine egg, Grade 4, in the first three generations. That is, the growth is better than Group 1, but not increasing any more quickly in the first five generations on glycerine egg. Glycerine potato slight or very moderate in the first four generations.

Group 3. — At first as in Group 1 * on both glycerine egg and potato, but rapidly increasing in amount in the first five generations on glycerine egg, so that the amount of growth on this medium reaches Grade 7. Pigmentation begins to appear on reaching the higher grades. It is then equal to the human type in amount of growth.

This grouping evidently gives us an almost unbroken series of types of growth. When we come to analyze numerically the cultures according to the groups into which they fall we shall see that this apparent gradation is not inconsistent with our general statement that the human type of bacillus is eugonic, the bovine type dysgonic, and that this difference alone makes one able in the great majority of cases to tell which type one is dealing with.

Classifying the cultures according to their source and the groups into which they fall gives us the following tables:

* This might read "as in Groups 1 or 2," in which case all possible variations would be included. We have had no cultures which would warrant this addition though there is a possibility that such may occur.

HUMAN TYPE. FROM MAN.

Group	I.	II.	III.	IV.
Number	387	13	2	2
Per cent	95% +	3% +	·5% —	·5% —

One case, general tuberculosis children, Nos. 288 and 289, showed difference in two strains, classified according to poor strain. Another case, general tuberculosis children, Nos. 634 and 651, was a double infection, one strain given in this table, one in the next.

BOVINE TYPE. FROM MAN.

Group	I.	II.*	III.*
Number	33	0	0
Per cent	100%	0%	0%

BOVINE TYPE. FROM CATTLE (FIVE FROM MILK).

Group	I.	II.	III.
Number	26	1	2
Per cent	89% +	3% +	6% +

BOVINE TYPE. (COMBINATION OF TWO PRECEDING TABLES.)

Group	I.	II.	III.
Number	59	1	2
Per cent	95% +	1% +	3% +

* The numerals in the case of bovine viruses do not mean that Group 3 is more atypical than Group 2. Group 2 is probably further from the type considering the early generations, but Group 3 comes close to some human variants showing reluctance for one or two generations.

When we examine these tables we find that the Human Type Groups 1 and 2 contain the large total of 98% + of the human viruses, and the Bovine Type Group 1 contains

95% + of the bovine viruses. In other words, over ninety-eight per cent of all viruses fall into the groups that are so distinctive as to be surely diagnostic.

Human Type Group 3 and Bovine Type Group 3, considering the early generations alone, are distinctive enough for diagnosis of type. This really leaves only two groups where any trouble was encountered and in these two groups fell only three or .6% + of the total number of viruses.

The preceding description covers the majority of viruses so well that a description of the individual cultures would be a continued repetition. With the great number of viruses we have studied this would also take too much space with no proportionate gain. We shall content ourselves with a description of the viruses showing variations from the extreme types. These cultures have been spoken of by different authors as atypical or irregular. As will be clear in the later discussion (page 106) of the place of these cultures, we should prefer to speak of them as viruses showing variant characters. The descriptions will also bring out some minor points which we have found useful in differentiation.

In the great majority of viruses the different strains from the same case, whether from the lung, spleen or other organs, have been uniform. Variations, however, have occurred, sometimes in duplicate cultures from the same organ or even in tubes of the same culture. These will be noted in the following descriptions:

DESCRIPTION OF CULTURES SHOWING VARIATIONS.

Pulmonary tuberculosis. Adults. — No. 76. H. type 2b. — Original cultures and subsequent transfers on egg typical of human type. Glycerine egg, not planted till fourth generation, growth typical. Potato, growth reluctant in early generations, Grades 0-2 at the end of three weeks. Longer incubation then tried and this gave sufficient growth for transfer and in the ninth generation, third on potato, it reached Grade 5, at the end of six weeks, with pigmentation. The fourth generation on potato reached Grade 8 in three weeks, as also subsequent transfers. Pigmentation was very constant. In the early generations the same reluctance was noticed with glycerine agar.

No. 286. H. type 1. — Original cultures on both egg and glycerine egg only showed a few small isolated colonies so that comparison and grading was not done. The first transfer on glycerine egg gave a typical culture, Grade 8, pinkish, as also subsequent transfers. The first transfer on potato from the poor tubes was negative, but made from the subsequent better growing egg tubes was typical. This strain simply gave poor growth on the original tubes, due possibly to few bacilli being present in the tissue inoculated. The guinea-pig was not very tuberculous.

No. 307. H. type 1. — Same as preceding, except that glycerine egg reached Grade 7. The guinea-pig was somewhat more tuberculous macroscopically.

No. 341. H. type 1. — Same poor growth in the first generation, *i.e.*, original cultures. Positive only on glycerine egg. Subsequent transfers typical. Guinea-pig showed moderate amount of tuberculosis, further advanced than in two preceding cases. Lymph nodes showed caseation.

No. 371. H. type 1. — Also showed poor growth in the original cultures. The first transfer on glycerine egg reached Grade 6, pinkish; the second transfer — the third generation — reached Grade 8. Except for this initial reluctance which showed somewhat in the second generation, the growth has been typical. The guinea-pig was rather markedly tuberculous.

No. 407. H. type 2a. — Growth typical except on potato. On this medium it has fluctuated between Grades 2 to 5, throughout the first ten generations. The growth on potato has shown a somewhat marked tendency to pigmentation in spite of its sparseness. Even in the first transfer to potato, though reaching only Grade 2, it showed reddish pigmentation.

No. 414. H. type 1. — Poor growth in original cultures, otherwise typical. Guinea-pig moderately tuberculous.

No. 421. H. type 2b. — Persistent poor growth on potato. Longer incubation, six weeks, gave only Grade 5. This is still unchanged at seventh generation.

No. 425. H. type 1. — Poor growth on the original cultures, but subsequent transfers typical. The first transfer from the poor tubes even on potato gave Grade 7.

No. 437. H. type 2a. — Poor growth on potato, but even in the second transfer on potato growth increased to Grade 7 with six weeks' incubation. Beyond this reluctance and slowness of growth on potato, the growth is typical.

No. 500. H. type 2a. — Growth on potato reached only Grade 5 in first three transfers, then increased on subsequent transfers to Grade 7. Otherwise typical.

No. 516. H. type 2b. — Growth on potato very poor, most of the transfers being negative at the end of three weeks, the highest being Grade 3. Growth on glycerine egg typical.

No. 517. H. type 2b. — Growth poor on potato, gradually increasing so that in the eighth generation, third on potato, reached Grade 6. Otherwise typical.

No. 539. H. type 2a. — Reluctant and slow on potato, Grade 5, the highest point reached in first four generations. On the fifth generation, Grade 3 in three weeks, Grade 7 in six weeks. Otherwise typical.

No. 545. H. type 2a. — Growth on potato only reached Grade 5 in first four generations. Otherwise typical.

No. 566. H. type 2a. — Potato, Grade 4 in early generations, Grade 6 in the sixth generation. Otherwise typical.

Tuberculous adenitis. Adults. — No. 619. H. type 1. — First generation very poor, as was also the first transfer to potato from these tubes. Otherwise typical and vigorous in transfers. Guinea-pig showed rather marked tuberculosis.

Tuberculous adenitis. Children. — No. 18. H. type 2a. — Potato poor in early generations, Grade 4, then irregular but capable of reaching Grade 7. Otherwise typical.

No. 94. H. type 1. — Poor in original tubes. Subsequent transfers typically luxuriant. Guinea-pig markedly tuberculous, and shows abundant bacilli in smears.

No. 670. H. type 1. — Poor in original tubes and first transfer from these to potato. Other transfers typical. Guinea-pig showed moderate tuberculosis.

Generalized tuberculosis. Children. — Nos. 1 and 45. H. type 2b. — Both were reluctant on glycerine agar in the early generations and also on potato as soon as tried. No. 45 has continued poor on potato; No. 1, however, has increased. In the ninth generation it reached Grade 8.

No. 276. H. type 1. — Poor on first generation. Subsequent transfers typical and luxuriant. Guinea-pig very moderately tuberculous.

No. 377. H. type 4. — Egg, first three generations, Grades 2 to 3. Glycerine egg, first three generations, Grade 4, including original culture which was Grade 4, the early generations showing some tendency to moistness. The subsequent range on glycerine egg has been from Grades 4 to 5, occasionally reaching Grade 6. The growth is dry, yellowish and spreading over the glass at the edges of the media. Potato and glycerine agar have usually failed to give growth and if there was any, Grade 3 was the highest it reached.

Correlated with the spreading on the glass is the pellicle formation in egg tubes containing bouillon which started in the third generation. The pellicle was good and in some instances thick. The noteworthy feature about this strain is the fact that there has been very slight increase in saprophytism even after ten generations.

No. 377a. Duplicate from second pig. Identical with 377.

No. 377b. Duplicate from pig injected with organs of pig of 377a. Identical with 377 and 377a except that glycerine egg ranges about one point higher in grade and reaching Grade 6 by the fifth generation.

The tendency to pellicle formation, the formation of a complete, somewhat crumpled or wrinkled layer spreading to the glass on glycerine egg media, made us consider this a human strain from the cultural characteristics. This was borne out by the virulence tests. The tendency to moistness, while not marked and having rather the appearance of having been moist and more or less dried down is unusual on glycerine egg with human viruses.

No. 389. H. type 2a. — Same virus as strain 388, H. type 1. The only irregularity is slow growth on potato, but with longer incubation able to reach Grade 8.

No. 434. H. type 1. — Showed poor growth in the original tubes. The increase was then more gradual than in any viruses showing this irregularity. Thus glycerine egg, original = Grade 3, generation 2 = Grade 3 +; generation 3 = Grade 8, pinkish. The transfer on potato from the original was poor, from the next generation it reached Grade 6. A few of the early glycerine egg tubes showed slight moistness.

This culture, then, fulfils the conditions of Human type, Group 1, but only fulfilled these conditions on the third generation.

No. 434a. — Duplicate from second pig. Same as preceding except that the third generation on glycerine egg was less (Grade 6 +), and reached Grade 8, pinkish, on the fourth.

No. 435 (see 434). H. type 1. — Same as 434 except that third generation on glycerine egg was Grade 7, pinkish.

No. 435a. — Duplicate from second pig. Same as 435 except that third generation on glycerine egg was slightly poorer, but the potato increased somewhat more rapidly.

In all these strains the guinea-pigs showed somewhat marked tuberculosis.

The chief point about these strains is their tendency to general uniformity in cultural characters. They have been placed in Group 1 on their general ability to fulfil the criteria laid down, but on the other hand they showed relationship to the succeeding groups in their initial reluctance of growth.

Tuberculosis of bones and joints. — No. 202. H. type 4. — Original cultures on glycerine egg, Grade 4, and subsequent transfers up to the tenth generation have fluctuated between Grades 4 and 6. The growth is confluent, spreading, and the early generations showed some tendency to moistness. Potato very poor in the early generations and later irregular, but on the twelfth generation reached Grade 9, far beyond the amount on glycerine egg. In the second generation glycerine egg tubes with bouillon showed good pellicles.

On the whole growth is better even in the early generations than general tuberculosis child No. 377, and less difficulty was experienced in concluding its cultural type.

No. 558. H. type 3. — Original glycerine egg tubes, Grade 4, then gradually increased in amount and finally reached Grade 8, pinkish in the ninth generation. The early poorly growing tubes showed some tendency to moistness of growth. Potato reached Grade 5 in the first four generations and finally by the tenth generation reached Grade 7. Pellicle formation was early (glycerine egg).

Duplicate from second pig. Same as preceding but slightly poorer. Has not been in cultivation long enough to tell if it will increase the same.

No. 592. H. type 1. — Poor in the original tubes, otherwise typical. Guinea-pig moderately tuberculous.

Miscellaneous. — No. 661. H. type 3. — The original glycerine egg tubes were Grade 5, subsequent transfers to the fifth have been Grade 6 with pigmentation marked in some generations. The potato was negative at first, reaching Grade 4 in the fourth generation and Grade 8 in the fifth, being better than on the egg. Pellicle formation early and marked (glycerine egg).

Viruses of bovine origin. Controls. — No. 314. B. type 3. — Original cultures on egg were Grade 2, and subsequent transfers on this medium remained the same for six generations. Original cultures on glycerine egg were negative. Transfers from the second generation on egg to glycerine egg and carried on on this medium gave the following results: The first two transfers, the third and fourth generations, were Grade 2. The next transfer, Grade 4, and on longer incubation showed a slight pinkish tint. Another transfer, the fourth on glycerine egg, but the sixth generation, equalled Grade 7 and showed distinct pinkish tint. In every way it resembled a fairly good growing human strain. The potato cultures show a more sudden rise. The first and second transfers only gave Grade 1, the next transfer gave Grade 7 with a suggestion of pink to the growth. The total generation of this culture was the sixth.

This bovine strain was therefore capable of adapting itself extremely rapidly to artifical media, quickly increasing in amount so that the amount of growth by the sixth generation was equal to that of a human strain.

A transfer to glycerine egg had been made from the original egg tubes, but had not been used. When the strain began to increase so rapidly, suspicious of possible error, we returned to these tubes and started another series. This series is now in the ninth generation, the eighth on glycerine egg, and has only reached Grade 4. We have, therefore, not only variant characters, but extreme variation in series of cultures in the same strain.

The different series as will be seen were virulent for rabbits. No evidence of accidental admixture by reisolation from rabbits using the method advised by Kossel, Weber and Heuss. The reisolations of both series after passage through rabbits and guinea-pigs showed no change.

No. 327. B. type 3. — The original cultures on glycerine egg were negative. The original cultures on egg were Grade 2 and increased to Grade 3 by the fourth generation on this medium.

Some of the original egg tubes were incubated for an additional three weeks and one of them showed a marked increase. With the experience of No. 314 we immediately made a separate series from this tube.

Transfers from this on glycerine egg passed from Grade 1 to Grade 6 by the fourth transfer, viz.: the fifth generation, and to Grade 7 on the next, showing pinkish discoloration. This again was a rapid increase equaling a good human strain by the sixth generation. The potato, however, remained persistently sparse, but glycerine agar grew well, being one of the very few instances where there was a discrepancy in growth on these two media.

The series from the other tubes reached Grade 4+ on glycerine egg by the tenth generation, and Grade 6 on potato on the eleventh generation. In this case potato has given better growth in the later generation than either glycerine egg or agar.

This strain then not only varied from the type but gave extreme variations in the same strain and unusual irregularities on different media.

No. 327a. — Duplicate strain from second pig. This strain showed a more gradual increase. Reaching Grade 4 of the fifth generation, four on glycerine egg, jumped to Grade 6 on the eighth generation and to Grade 8 on the ninth. There was pinkish pigment at the higher grades. The ninth generation, being the third on potato, showed Grade 6 for this medium. The growth on bouillon and agar was also fairly good at this stage. This strain then increased more slowly and would be B. type 1 according to our classification. In this case the passage through the guinea-pig of the good-growing series gave a poor-growing strain.

No. 334. B. type 2. — This virus showed better growth in the first three generations than any other bovine virus encountered by us. Glycerine egg was negative in the original tubes, plain egg, however, reaching Grade 4. The subsequent transfers to glycerine egg gave Grade 4 on the second and third generations. The subsequent course is the same as in B. type 1.

Some of the more poorly growing tubes of the original culture were made into a separate series, but although they gave poorer transfers, probably due to the amount of growth, they gave no promise of marked variation and were dropped out of cultivation.

Passage through rabbits and guinea-pigs gave a slightly poorer growing strain, but the ability to grow immediately on glycerine egg was retained.

Descriptions of unusual viruses. — The details of the following viruses are given not because they are in any way irregular but being unusual the evidence is given in full:

GENERALIZED TUBERCULOSIS — CHILDREN. (TWO TYPES IN ONE CASE.)

No. 634. B. type I; meninges.	Glycerine egg, original cultures, Grade 1, slow increase to Grade 4, by the fifth transfer on this medium. Egg, original cultures, Grade 2, same for four transfers. Glycerine potato, first growth in the sixth generation, Grade 1, next transfer Grade 3.
No. 634a.	Duplicate from second pig. Glycerine egg, original cultures, Grade 1, slow increase to Grade 3 by the fifth transfer on this medium. Egg, grading doubtful, possibility of contamination. Glycerine potato, positive on the fourth generation, next transfer gave Grade 3.
No. 651. H. type I; mesenteric node (see 634, same case).	Original culture, egg, Grade 5, glycerine egg, Grade 8, pinkish. Glycerine potato, transfer from original tubes, Grade 6, reddish.
No. 651a.	Duplicate from second pig. Original culture, egg, Grade 5, glycerine egg, Grade 8, pinkish. Glycerine potato, transfer from original tubes, Grade 6, reddish.

GENITO-URINARY TUBERCULOSIS. (BOVINE TYPE IN ADULT.)

No. 199. B. type I; kidney. Adult.	Egg, original cultures, Grade 3, same on next transfer. Glycerine egg, original culture, Grade 3, remaining about the same, increase very slight for five transfers on this medium, Grade 4 being the highest. Glycerine potato, growth on the third generation, Grade 2, same for five subsequent transfers.
No. 199a.	Duplicate from second pig. Egg, original cultures, Grade 3, five subsequent transfers, Grade 2. Glycerine egg, original cultures, Grade 0, transfer from original egg, gave Grade 2, increase to Grade 3 by the fifth transfer on this medium. Glycerine potato very reluctant, reaching Grade 2 on the eighth generation, nearly all the earlier trials being negative. This strain, 199a, differed from 199 in failing to grow on glycerine egg from the start and the growth on the whole was poorer.

VIRUSES FROM MILK.* (HUMAN TYPE IN MILK.)

No. 111. H. type 1.	Glycerine egg, original culture, Grade 7. Egg, original culture, Grade 5. Glycerine potato, first transfer from second generation on glycerine egg, Grade 8, reddish.
No. 111a.	Duplicate from second pig. Glycerine egg, original culture, Grade 6, next transfer, Grade 7. Egg, original culture, Grade 4. Glycerine potato, first transfer from original glycerine egg, Grade 7.

* Isolated from guinea-pig inoculated from tuberculous pig injected with milk by Dr. Hess.

Cultural characteristics after passage through animals. — The Royal Commission has pointed out that a bovine culture having attained a certain luxuriance on artificial media tends to keep this luxuriance after passage through the body of an animal. In certain instances where there were variations in growth we have reisolated cultures from inoculated animals to see if this variation was constant. While doing this we have found that the observation made by the Royal Commission is in general accord with our own more limited number of experiments. Four bovine viruses were reisolated. In one the vigor of growth was unchanged. In the second, Bovine Virus No. 334, there was some loss in total vigor, but the ability to grow on glycerine egg was not lost. The reisolated strain approximated the poorer growing series made from the original strain. The same condition, though much more marked, was present in Bovine Virus No. 327. Here also the good growing series on passage through a guinea-pig reverted to the type of growth shown by the poor growing series. The last case, Bovine Virus No. 314, in two instances was reisolated by guinea-pigs from rabbits, one of which had been inoculated intravenously, the other subcutaneously with the vigorous growing series. It was also reisolated after injection into a single pig. In all instances the vigor of growth was unchanged. In the only instances, therefore, where there was change the original cultures showed like differences and may be construed as evidence of

instability in these viruses. They cannot be considered true exceptions to the general results of the Commission.

Reisolations of the human viruses showing variations culturally have given us strains identical with the originals.

Glycerine bouillon. — This medium has attained a good deal of prominence as a differential medium due to the work of Kossel, Weber and Heuss. Their results have been already given. Exception has been taken to their observation by others.

We have tried many of our cultures on glycerine bouillon and have come to the conclusion that the amount of difference in growth is inversely proportional to the number of generations which elapse before one has the skill or good luck to get satisfactory growth. If one reads what was done to insure growth by the workers in the *Gesundheitsamt* there is nothing more needed to convince one that this method is very unwieldy. Thus Oehlecker says, that before use one must be sure that the bouillon is fitted for the growth of tubercle bacilli and that they reserved fifty to seventy liters of one bouillon which had proved good to insure uniformity of results. This uncertainty in medium, the difficulty of the method, especially with bovine cultures, where sufficient pellicle for inoculation is obtained only with much trouble and manipulation, the impossibility of comparing like generations and growth for identical periods of time, the tendency to sink, or of edges to be submerged, shown by the thin bovine pellicles on handling the flasks, the amount of space and the time for incubation with a large number of cultures; all these difficulties became so prominent in our work that we soon ceased using it. The greatest difficulty, however, is that, after a few failures with a bovine virus, reinoculation must necessarily take place from growth on other media. This means that the strain is no longer in the early generations and that the growth may be good on bouillon because the strain may already have lost some of its dysgony, which would be especially true if the strain had become accustomed to glycerine.

Our results, so far as growth was compared in sufficiently early generations, are in accord with conclusions given by the workers in the *Gesundheitsamt.* One conclusion seems absolute, viz., any virus forming a complete wrinkled moderate or thick pellicle in the first three generations is surely of the human type. If we compare this, however, with our results on potato, we find that this medium, in practically all cases, gives us the same information, and avoids all the difficulties found in bouillon except irregularity in different batches of media and this can be reduced to a minimum. The only possible exception would be that where potato gave poor growth, as with a few variant strains, possibly bouillon would give more marked differences in these cases than potato, but even in these cases, with the exception of a very few, the glycerine egg settles the question, and potato or bouillon only serve as an index of the degree of saprophytism, and as contributory evidence.

Some of our early failures to get pellicle for bouillon work were somewhat peculiar. Glycerine egg and egg with glycerine bouillon added so that about one-third of the surface of the slant was covered were used to get pellicles of human viruses. They proved extremely efficient. It was hoped that bovine viruses, possibly a little later than the human viruses, would start to spread on the bouillon. We could not get this result in any of the early bovine cultures. The growth would be moist, at times even somewhat slimy, or in other instances more dry, and at the edge of bouillon tend to heap up somewhat, and although sunken floccules would be found or even occasional floating specks, a complete pellicle however was not formed. Thinking that the routine three-weeks' incubation was too short we incubated for longer periods with no better results in obtaining a complete membrane. It was only later when the growth increased in amount that pellicle formation was successful. This result is in striking contrast to the results on glycerine potato and agar, where in a certain percentage of the cultures thin pellicles will spread over the water of condensation very early. With the former medium we have seen this on the second

generation. These early pellicles were very thin and very difficult to transfer.

The reason for this discrepancy we have felt is due to the marked tendency to moistness of growth on glycerine egg, which though somewhat evident on other media is not as marked. Whatever the reason, the fact has bearing on the differences noted on glycerine bouillon itself.

This observation appears to us as an unconscious verification of the work of the *Gesundheitsamt.* Even in the strains which we have placed in human type groups 3 and 4, this faculty of early pellicle formation on glycerine egg media was marked, and good pellicles were formed. On the other hand, with bovine viruses where pellicles were obtained in the early generations by using potato or agar, the pellicles though completely formed were extremely thin. For this reason we have found the glycerine egg, with bouillon added, a valuable accessory differential medium in cultures tending to vary from the extreme type on glycerine egg and glycerine potato. If it is used, many tubes should be inoculated, every precaution taken against evaporation, and tubes incubated in the inclined position to increase the surface of bouillon.

With these facts and the distinct statement from the *Gesundheitsamt*, with which we agree, that the growth on bouillon is only diagnostic in the early generations, makes us doubt whether dissenting opinions were always founded on evidence obtained with this precaution in mind. The further criticism of variability of growth we find to be beyond the point. With a variable medium large numbers of tubes or flasks should be used and only the best growth considered typical of the capability of growth of the virus. The poorer growths are not evidences of inconstancy of the virus in this case, but evidence of inconstancy in favorable conditions.

If we now add this factor and examine the protocols of our viruses classed as variants on the amount of growth alone, we find that this completes the separation of the two types, and where we were in doubt this has helped us to our final conclusion.

This may fail us just as the bouillon does, because of the same reasons probably, and as conclusions should only be drawn in the first few generations to be safe, we prefer to consider it an accessory rather than the main method of differentiation. Failure is only evidence when all the other growth characteristics are in accord, success is only of value where one is in doubt; where the growth is typical one has the information already.

Glycerine agar has the same serious objection as a routine medium as bouillon — variability. If the time of growth be limited it gives us the same evidence as potato. Very occasionally it has given growth when this medium failed, but too infrequently to detract from the general uniformity of potato. It is very useful to get a pellicle or scale with bovine viruses for the inoculation of bouillon.

Our conclusions again stated are: All viruses when classified according to their most constant difference, amount, and rapidity of growth, fall into two groups with a small percentage of variants in each which tend to bridge these groups. These groups have in all cases been in accordance with final conclusions as to type, that is, one group contained the viruses of the human type, one the viruses of the bovine type. The viruses, which are classed as variants, were in nearly all cases sufficiently characteristic to be separated and, with the added factor of pellicle formation, the presumptive classification of these variants was completed. This grouping has been confirmed in every way by the subsequent virulence tests.

Correlation of results. — If we compare our conclusions as to cultural characteristics with those of previous investigators our results are similar if all factors are considered. The general fact of the greater luxuriance of growth of the human type practically agreed upon by all has simply been elaborated by us to the last degree. The differences noted by Dorset and others on egg media without glycerine have practically been duplicated. This difference, however, was not followed after more marked methods of differentiation

with glycerine egg media were found. Pigment production as already stated has been found to be valueless as a means of differentiation.

The clear cut differences as to growth on bouillon noted by the workers of the *Gesundheitsamt* has received confirmation. We feel, however, that this method is cumbersome for the reasons already stated, especially when simpler methods are available. However, the conclusion we reached that any virus which quickly forms a vigorous pellicle on bouillon in the first few generations is without the possibility of doubt of the human type is completely in accord. Further, our observations of pellicle formation on other media indirectly verify their results. Their conclusions seem extreme and their method of statement so sharp that on the surface one would assume that all cultures of one type were identical. This seems to have been the point of view of those who have tried to controvert their results. What seems more likely is that they were insisting on the differences between these two types and were less interested in the variations shown by individual cultures. Because of the criticism of this work we have paid a great deal of attention to the variations in individual cultures to see if they impaired the differential evidence to any extent. This we have found not to be the case.

Passing to the results of the Royal Commission, we find a far closer parallelism of results than is evident on the surface. Only when we go further than the report and examine both the opinions of the individual workers and the protocols given is this evident. We think that had their results been always based on observations of earlier generations and more comparable to our own, the results would have been nearly identical.

Cobbett's conclusions are as follows:

(1.) "Tubercle bacilli from man fall into two groups which present fairly distinct characters of growth."

(2.) "These characters are determined by quantity and rate of growth on certain culture media. The ultimate quantity appears to me the more significant and useful."

(3.) "It is on media containing glycerine that the difference becomes marked." *

(4.) "The two cultural groups into which human tubercle bacilli fall may be defined as including:

"1. All those strains the growth of which in early cultures is little if at all aided by glycerine.

"2. Those which grow much more luxuriantly on media to which glycerine has been added."

On further discussion of this method of differentiation he says that the tendency of the two types to approach was, for the most part, due to the fact that the strains were not strictly comparable, as they had been in cultivation for very different lengths of time; the comparison being better the shorter the time they had been in cultivation. "If primary cultures could always be used for comparison, I believe that strains with intermediate characteristics of growth would to a large extent disappear and each group contract and become more sharply distinct from the other." Further, "after one had gained some experience one seldom, if ever, was unable to foretell the comparative virulence of a strain from a cursory study of the characters of its early cultures." A mistake was made only once and then was due to an unsatisfactory batch of media. "Only the best growths on a given medium should be considered characteristic."

These conclusions coincide in every way with our own, except that we prefer to set a time limit for growth, that is, to consider rapidity as well as amount.

According to the conclusions of Eastwood, whose method of classification was used mainly by the Commission, the results are different.

He classes all cultures into five groups according to the total growth on glycerine agar, potato, and broth and finds an almost complete series of gradations in growth from the

* Significant in connection with our own findings, he says, "It is perhaps more marked on glycerine agar and glycerine broth, but these media are apt to be uncertain and growth on them at best is slow. Glycerine serum appears to me to be more certain, and on it results are more quickly obtained."

most luxuriant to the most sparse. Considering only his table of cultures from original material or least removed from the original material, we find that there is no uniformity according to the protocols as to the age of cultures received for examination. Further, one cannot be sure how soon thereafter the observations were made. Assuming that the observations were made on the very next generation after receiving a culture, which is not likely, his results show more variations than we have found and more cultures are placed in the groups which approach each other. Whether this is due to variations in the ages of the cultures or due to the method employed we are unable to say. In so far as variations and the tendency of the two types to approach each other culturally are concerned we are in agreement. The proportion of cultures, however, showing intermediate characteristics is far greater than among ours and more than one would expect with the results of Cobbett's examination of many of the same cultures.

The results of Lydia Rabinowitsch follow very closely those of Eastwood. It is her work which, more than any other, has accentuated the variations in cultural characteristics and led to the assumption of atypical cultures. Her description (1906) of her cultural work shows conditions that were far from ideal. She failed to find Dorset's egg medium of much value, which contrasts sharply with the results of others who have found it to be the medium above all others for isolation, especially for bovine cultures. Further, her serum medium could not have been very favorable when she found potato a more reliable medium for isolation. For this reason where she finds cultures atypical in that they did not grow well at first, we are inclined to think that this is referable to her media. Further, where different types of growth occur in the same transfers, the best growth is the typical one, the others simply show that conditions were not the best, due to variations beyond control. Even the two bovine cultures (Schakal and Spiesshirsch) which grew well from the start, she notes that although growth on bouillon was rapid it was not as

luxuriant as the human type and developed wart-like thickenings only very slowly. Other cultures belonged to one type or the other and showed sharp differences.

The above descriptions give a sufficiently clear idea of the relation of our results with those representative of all grades of opinion. We feel that our results are in essential accord with them all and that where differences occur they are differences of degree. That there is practically as much difference shown among extreme cultures of one type or the other as between the two types has no bearing in our mind on the point at issue, as the cultures of the two types do not overlap in characteristics. The question is, are there two groups into which all cultures from man and cattle fall, and is the one group from man identical with that from cattle and is its range of variation the same? To this we answer unreservedly, yes. It is too much to expect stereotyped results in either group. Biological reactions, especially with organisms so very closely related and probably originally from the same ancestor, would be expected to show some relationship. The number of viruses showing intermediate characteristics will vary with the technic employed. Our experience, as well as that of others, has shown that the simpler the methods the more ideal the medium, and the greater the number of tests to avoid irregularities not under our control but dependent on the material employed, the greater will be the difference shown between the two types and the smaller the number of cultures showing intermediate characteristics.

MORPHOLOGY.

Resumé. — Smith in his first communication found that when grown on serum the bovine type of bacillus is short (one micron), approaching oval cocci and deeply stained, whereas the human type is longer (about three micra), slender, curved, and decolorizes more easily. This difference is more striking on further cultivation. His later work confirmed these differences. Ravenel's findings are similar.

Dorset noticed no marked differences on his egg medium,

generally speaking all individuals were well stained, short, and rather thick.

Wolbach and Ernst found practically no differences on egg. On glycerine agar and serum there was an average shorter length for the bovine. On bouillon both showed relatively long forms, the human tending to be longer.

Kossel, Weber and Heuss found morphological differences on bouillon cultures, the human type being slender, mostly of regular shape and evenly stained; the bovine being thicker, shorter, irregular in shape and unevenly stained. The latter often showed clubbed or granular forms which made one think of diphtheria bacilli, that is, the bovine type showed pleomorphism. This difference is only evident in freshly isolated cultures and cultures grown under identical conditions.

On serum the Royal Commission found the bovine type fairly uniform with an average length of one micron, or a little less, that is, short and nearly all straight and uniformly stained. On other media they are irregular, broadly in proportion to the luxuriance of growth. The human type on serum vary from .5 to two micra and are straight and uniformly stained. In other media they are longer on the whole, varying more in length, less regularly straight and less uniformly stained than the bovine type.

Rabinowitsch, Fibiger and Jensen and others have found these general differences, but consider them too inconstant to be of help in the differentiation of type.

Own observations. — Cultures have been examined at various generations, and while there is a tendency of the human type to show longer forms and for the bovine type to show shorter forms, in the later generations, this has not been true in the first generation on glycerine egg. Likewise, while there is a tendency after cultivation for some time on this medium to show differences in staining, the bovine type being evenly stained more commonly and the human more unevenly stained, this does not appear at first.

In general this tendency to differ morphologically is much

less marked than cultural or virulence characteristics. While we have examined various generations on the different media used, as plain and glycerine egg, glycerine agar, glycerine potato, and glycerine broth, none showed the appreciable differences of the glycerine egg which corresponds closely with the serum media of other workers. The following approximate percentages of cultures examined at different generations based on preponderance of long or short forms, and even or uneven staining, will give a clear idea of the degree of differences.

In the first generations on glycerine egg from original material the irregularity of the organisms in form and staining reaction was so marked that it was impossible to judge to which type the virus belonged. Many of the human strains showed short, evenly staining forms, and the bovine on the other hand had bacilli of such length and uneven staining that they would be ordinarily classed with the human type; however, in later generations, as seen below, the differences became more apparent.

Fifth Generation:

Bovine type, 50% short, 50% medium, 0% long.
Human type, 17% " 50% " 33% "
Bovine type, 75% evenly stained, 25% unevenly stained.
Human type, 20% " " 80% " "

Tenth Generation:

Bovine type, 66% short, 20% medium, 14% long.
Human type, 16% " 25% " 59% "
Bovine type, 71% evenly stained, 29% unevenly stained.
Human type, 21% " " 79% " "

The above figures show that morphologically there is only a relative difference and this only in a certain proportion of cultures. There is no tendency in this characteristic to show two extreme types around which most viruses group themselves with only a small percentage of viruses tending to approach from these extremes as shown in cultural and virulence observations. In fact, the whole tendency morphologically is for a smaller percentage to fall into two different groups and for a larger percentage to fall between, forming a

complete and gradual gradation of one type into the other. While one can often by inspection of a slide make a tentative diagnosis of the type, the number of intermediate gradations in morphological differences rob it of nearly all practical value in the differentiation of type. This is the only characteristic in which there is overlapping when the cultures are grouped according to the gradations from the extreme typical groups. It is here that one would on general principles expect the least difference.

We conclude, then, that although morphological differences appear after cultivation on glycerine egg these differences are not sufficiently constant to be of practical value in the diagnosis of type.

Correlation of results. — As our observations have been on a different medium from that used by others the results are not strictly comparable. They follow closely the results on serum, however, although probably less marked in the early generations than on this medium. Comparing the results with those on plain egg, which we also found of no value, it is interesting to note the difference caused by the presence of glycerine.

We have been unable to compare our results on glycerine bouillon with those of the *Gesundheitsamt.* Although we found nothing of value our cultures were not examined under identical conditions, the greater number of the cultures being in cultivation for some time.

The majority of observers, however, have not placed much emphasis on these differences and have mainly relied on cultural and virulence tests for practical differentiation, which coincides with our own views. On the other hand, some have advanced the morphological resemblance as evidence of identity or called viruses intermediate or atypical because of this resemblance, regardless of the other characteristics of the culture examined. Such views necessarily in no way coincide with our own.

RABBIT VIRULENCE.

Resumé. — Early investigators had noticed differences in virulence of tuberculous material from cattle and man, although they did not appreciate the significance of this difference. Thus Villemin in 1868 said: "Nous ferons remarquer qu' aucun de nos lapins, inoculés avec du tubercule humain, ne nous a présenté une tuberculisation aussi rapidement et complétement généralisée que celle que nous avons obtenue avec l'inoculation du tubercule de vache." Other investigators previous to the discovery of the tubercle bacillus also noticed this difference. Orth found that feeding tuberculous human lungs to rabbits gave no results, whereas the feeding of bovine material practically always caused generalized tuberculosis. The inoculation experiments of Baumgarten gave the same results, the bovine material only causing generalized tuberculosis and this with "mathematical certainty."

Smith in 1896 was the first to attempt to correlate and use this difference in virulence in rabbits with the other differences noted in tubercle bacilli of human and bovine sources, and added further observations in 1898 of the value of this evidence as a differential method.

Vagedes, in 1898, reported that tubercle bacilli fell into three groups according to their virulence for rabbits. Although this classification is no longer maintainable, his work is of value as he laid the foundation for the methods of inoculation used later. His work clearly showed the virulence of fresh bovine viruses, which were found capable of causing generalized tuberculosis even in very small doses. The amount inoculated was estimated by weight.

The work of Kossel, Weber and Heuss settled many of the points of value, and they concluded that the differences in virulence in rabbits runs parallel with those found by inoculating calves.

Nearly all of the subsequent investigators agreed to this difference, though some found irregularities in results. These irregularities were probably dependent on dose and method of inoculation. As Oehlecker has shown, the smaller doses

give the greater differences. This is especially true of the intravenous method of inoculation.

Own observations. — For reasons of convenience the intravenous method of inoculation was selected. The possibility of sinus formation with discharge and contamination of the surroundings kept us from adopting the subcutaneous method. The intraperitoneal method would not have this objection, but there was danger of injection into the intestine or the greater danger that with the development of local tuberculosis the marked tendency to localization in the lungs shown by rabbits regardless of the place of inoculation, would give apparent generalization and spoil any contrast. The intravenous method as far as we could tell from the work of others would give the quickest results. This factor more than any other caused us to adopt this method.

Rabbits of varying weights have been tried. At first we used only rabbits between one thousand five hundred to two thousand grams. With the difficulty of obtaining animals necessary for the great number of tests, we have had to use rabbits of still smaller size. We cannot see that this variation in weight has made any difference. In every case the virus which had been tentatively classified as of bovine type had light and heavy animals in the series. The human type was injected into the smallest rabbits whenever these had to be used.

The material for inoculation was from egg cultures, with few exceptions, and usually from tubes to which glycerine bouillon had been added. The growth just above the bouillon was found to be more easily subdivided and formed better suspensions. The transfer used varied in age from twenty-one days to one month. The growth was removed with a platinum spatula to smooth surface filter paper and, by smearing it on the surface somewhat as one would spread an ointment, the excess of fluid was quickly absorbed. The mass was then transferred to fresh filter paper and weighed. After weighing, the mass of culture was removed to a conical glass, the paper and remaining culture weighed, deducting

which, gave us the weight of the culture to be used. A glass rod was then dipped into sterile saline, the adherent drop giving the right amount of fluid to start the subdivision of the culture mass. With this rod the culture was thoroughly ground up and saline gradually added, so that each cubic centimeter represented one milligram of culture. In each case one cubic centimeter was injected, and where 1/100 milligram was used the suspension was diluted accordingly. In some instances the amount of growth of a bovine virus was insufficient for weighing. In this case a suspension was made and approximated as closely as possible to a suspension made from weighed tubercle bacilli. So that the error, if any, should fall on the lesser side, a few cubic centimeters of saline were added in excess. This method has given us results approximating very closely emulsions made from weighed cultures. Two or three exceptions have occurred. In these cases death was delayed as shown by subsequently injected rabbits receiving weighed amounts. In one way this approximate dosage is very efficient, viz., it allows the testing of viruses even in the first transfer from the original cultures. As we shall see, the earlier the test is made the more valuable it is.

The weight of the rabbit was recorded and also his weight at death. In the case of rabbits injected with bacilli of the human type, the weight was usually taken once in the interim. The dose used has been either one milligram or 1/100 milligram or both amounts.

When we started we were doubtful as to what criterion should be adopted. Should the results rest on inspection of the macroscopic lesions alone, or should microscopic examination and search for the bacilli be also employed? The latter would entail a large amount of work and to our mind had certain theoretical objections. First, the presence of microscopic lesions might confuse rather than enlighten one. As far as we could see, the main thing to consider was the ability to produce *progressive* lesions. Thus, our observations led us to the general opinion that lesions from viruses of low virulence retrogressed; the average lesions in rabbits

dying after a short period were much greater in number than in those gaining weight and killed after long periods. Second, if microscopic examination was to be used it should be used for all, and for all organs routinely examined in the macroscopically negative as well as in the macroscopically doubtful cases. If examinations for bacilli were made the same routine should be followed. As we were able to isolate bacilli, as were also others, from macroscopically unaltered organs, we felt that the occasional examination of doubtful lesions gave us no absolute information, unless the negative organs of other rabbits were also routinely examined for tubercles and bacilli, as controls.

Because of these reasons and the clearly cut results given by our early experiments, we decided to observe only the macroscopic lesions. Thus only the ability of a virus to cause progressive lesions was considered and the presence, number, and distribution, viz., degree of generalization of the macroscopic lesions, was assumed as the index of this power. At times this has left us with doubtful lesions which had to be interpreted one way or the other, but even with these lesions the remaining ones gave the answer. Wherever doubtful lesions have occurred, raising the question of the presence or absence of generalization, the other rabbits of the series or subsequent controls cleared up this point.

Certain details of rabbit pathology are very important. There are a few diseases in rabbits which give rise to lesions in rabbits, the nature of which, tuberculous or not, is at times difficult to decide. Without a knowledge of these, many lesions would be called tubercular which had no etiologic relation to tuberculosis. Further, the injection of tubercle bacilli seems to aggravate these diseases or render the rabbit especially susceptible to them. This leads to the fallacy of assuming death to be due to tuberculosis, where this is not the case and if the fact of death alone be used as an essential point, there results a great deal of confusion. It is not always possible to say what caused death, but if one compares a rabbit with generalized tuberculosis, having very extensive lesions and not losing ground very rapidly, with a rabbit

dying in from two to four weeks with a few lesions in the lungs, one can be sure that tuberculosis was not the direct cause of death in the latter case. With such moderate lesions, the majority of rabbits gain weight and live indefinitely. The toxic effects of the injected virus may cause death in a short time, but in those dying later the injected virus acts as a predisposing cause, injuring the resistance of the rabbit. Death cannot be considered as due to tuberculosis alone unless the extent and severity of the lesions are considered.

The post-mortem lesions giving the greatest trouble are those of coccidiosis, rabbit septicemia, and abscesses, subcutaneous or of the lymph nodes, following bites. Oehlecker has given the best account of these lesions and their relation to tuberculosis. Coccidiosis leads to trouble only when in the liver. The majority of coccidial lesions are easily distinguished. In the liver they are irregular in size and of a yellow color, or in old lesions give the appearance of connective tissue scars. When, however, they are very small and the color therefore not so easily recognized, they lead to difficulty. If a liver containing doubtless tubercles is used for comparison, there are very few instances where one cannot conclude at once the nature of the lesion. On the other hand, the presence of coccidiosis may obscure the tubercular lesions which are present and render it difficult to estimate their extent. The coccidial cysts and enteritis give no trouble.

Rabbit septicemia is accompanied by a fibrino-purulent exudate of the serous surfaces, especially of the pleura. The exudate has nothing to do with tuberculosis. Ignorance of this fact leads one to assume a like relationship of this lesion in man and the rabbit. With the fact in mind, there will be no trouble except that, when rabbits are badly infected, tubercular lesions may be obscured.

The results of infections from wounds may be very severe and widespread subcutaneous abscesses may follow. The character of the lesion is usually evident. At times, however, the contents may simulate caseous material very closely and leave one in doubt. The infections of the lymph nodes often

simulate a caseating adenitis. Although we constantly kept this condition in mind to avoid mistaking these lesions, there were many cases where we considered the lesions tuberculous at the time and later had to conclude that they were not or were very doubtful.

All subcutaneous abscesses, which are commonly widespread, are not tuberculous, however closely their contents may simulate a cold abscess. We have encountered these lesions in rabbits dying in such a short period after inoculation as to preclude any possibility of the development of a cold abscess of this extent. These abscesses occur in the inoculated animals showing not the slightest trace of tuberculosis elsewhere. This is not conclusive, as the rabbit was inoculated, but is confirmatory evidence.

Associated with these abscesses there may be involvement of the lymph nodes. Thus, as an example, in a rabbit showing no lesions in any of the internal organs, the following is recorded: "Extensive subcutaneous (cold) abscess, involving both axillary regions and diffused over the abdomen as far as the tunica of the testicle. Left axillary node, caseous tubercles, right enlarged. Inguinal nodes congested." In other words, this condition simulated a tuberculous condition so closely as to be called tuberculous in spite of other considerations. Conditions interpreted as mastitis and considered at times to be tuberculous, probably belong also to this category. There may be an associated axillary adenitis simulating tuberculosis. Oehlecker mentions tuberculous mastitis, but whether he makes this diagnosis on microscopic examinations we do not know.

This leaves a certain number of cases of lymph adenitis for which no cause can be discovered. As we shall see, the bovine type is especially prone to involvement of the lymph nodes. Since this involvement is usually of many sets of nodes no doubt arises as to its nature.

Another common lesion in rabbits is orchitis. Here again we took it for granted that this lesion was tuberculous. When, however, a rabbit dying nineteen days after inoculation shows no tuberculous lesions, and has a marked

orchitis, with an abscess extending upward from the testicle to the liver, one has to revise one's opinion. A similar orchitis was found in a rabbit surviving only fourteen days.

Other lesions of like character have been found very occasionally involving bones or joints. Whether these are tuberculous or not is also somewhat doubtful.

Wherever smears have been made from these lesions no tubercle bacilli have been found. Unfortunately it was our late observations which brought doubt as to the tuberculous character of these lesions and it was no longer possible, because of lack of further material, to examine these lesions in section to determine their character. Fortunately, however, they have little importance. The question of generalization or non-generalization rests on the distribution of the lesions in the internal organs. What percentage of these accidental local lesions, if any, are tuberculous is of academic interest but not of practical value in differentiation. We have considered them at length so that they should not be called tuberculous without further evidence and used as indications of generalization where such is not the case.

Oehlecker called attention to tubercle-like lesions found in the blind portion of the intestine. These lesions are very similar macroscopically and microscopically to young tubercles. They are probably not tuberculous. We have examined them microscopically but can add nothing to what Oehlecker has found, but agree with him as to their exceedingly close resemblance to tubercles.

Broadly stated then our conclusions are: The bovine type of tubercle bacillus in every instance causes a generalized tuberculosis in doses of .01 milligram intravenously, the tuberculosis being progressive and causing the death of the animal. Human viruses injected in the same amount produce no disease at all or lesions of varying severity in the lungs or kidneys or both, never causing a progressive generalized tuberculosis. Even with one milligram, that is, one hundred times as much, the lesions are usually confined to the same organs and, though a very slight tendency to generalization is shown, there is never a generalized tuberculosis showing a marked progressive nature. The rabbits

injected even with the larger dose live indefinitely and, if death should occur, the tubercular lesions are usually not extensive enough to say that the animal died of this disease.

A rabbit inoculated with 1/100 milligram of tubercle bacilli of the *bovine type* gives the following lesions on autopsy. The superficial lymph nodes of the axilla and inguinal region show small tubercles or large tubercles with beginning central caseation. These lesions are almost pathognomonic. The lungs are literally a mass of tubercles, and one wonders how the animal survived so long. The tubercles are either small and closely packed, or larger and confluent with caseation in the center. The bronchial lymph nodes show tubercles. The heart as well as the diaphragmatic pleura may have tubercles. Commonly, tubercles are visible in the rib marrow. The spleen is enlarged, sometimes enormously, and usually thickly set with tubercles; occasionally they are few in number. The liver is commonly peppered with small grayish tubercles. The peritoneum, intestines, and abdominal lymph nodes may be involved. The whole picture is that of a widespread, progressive, generalized tuberculosis. The size of the lesions, confluence and caseation are dependent on the acuteness of the disease. This dose leads to retardation and gives the more distinctive picture.

If one milligram be injected, death is much more acute. The general picture is the same, but the lesions are very small and therefore not accentuated to the same degree as those in animals dying less acutely. Furthermore, death may be so acute that there is little visible, except very extreme involvement of the lungs. As we shall see later this is a serious objection to the use of this dose.

With the inoculation of 1/100 milligram of tubercle bacilli of the *human type*, the main tendency is for localization in the lungs. Here the bacilli are filtered out and show no further extension. The kidney is also commonly involved. A small proportion of rabbits show no macroscopic lesions at all. The appearance of the lungs varies. There may be a very few scattered tubercles in the lower lobes, or more in number and scattered throughout. The size also varies as

well as the amount of central caseation. Confluence may be present and, if associated with caseation, small ragged cavities may result. Occasionally the lungs are voluminous and severely involved with numerous confluent tubercles giving a picture like that of a rabbit infected with a bovine virus. The appearance of the kidney varies, the number of tubercles may be few or many. In some instances, after a long period, they may be the size of small peas and give a nodular appearance to the organ. Occasionally there is caseation in the pelvis. The majority of animals show involvement of both lung and kidneys, though only one organ may be involved. The only exceptions to this have been four rabbits: one showed two tubercles in the spleen, another showed tubercles in the intestine. Two rabbits showed apparent macroscopic tubercles in the superficial lymph nodes.

If one milligram be used the lesions are about the same, possibly a little more marked. With this dose, however, some generalization or involvement of other organs occasionally occurs.

The length of life following the injection of a human virus is indefinite. Where death occurs it is exceptional that the tuberculous lesions could be considered the cause of death. The general assumption that, if the rabbit survives the initial toxic effect (most marked with one milligram), no human virus is capable of causing a tuberculosis extensive and progressive enough to regularly cause the death of the rabbits inoculated seems justified.

If the milligram dose is used for differentiation certain precautions are necessary. The tendency of the bovine virus to kill so acutely without evidences of complete generalization, and the tendency at times to some generalization with human viruses makes this necessary. If death is uniformly caused by a virus in less than thirty days and if evidence of generalization is absent only in the rabbits dying in less than twenty days, the virus is of the bovine type. If, however, a rabbit survives an injection fifty to sixty days, with no lesions except in the lungs or kidneys or even with a few scattered lesions elsewhere, the virus is of the human type. An added point of verification in this case would be

gain in weight. If a rabbit dies in a period of time between thirty and sixty days and does not show the presence of complete generalization, one may fairly safely draw the conclusion that it was not due to the bovine type. The occasional failure, however, to show macroscopic evidence of generalization after the injection of one milligram of a bovine virus makes a longer period of life, namely, sixty days or more, a safer criterion with human viruses. This period of survival is much longer than that of any rabbit receiving a bovine virus. Rabbits dying at the period between twenty to thirty days are inadequate for differentiation unless generalization is very marked.

The time limit is also to be considered in the smaller dose; that is, the lesions in rabbits inoculated and dying of an intercurrent infection cannot be used as evidence against the ability of a virus to cause generalization, unless the rabbit lives long enough to allow evidences of generalization to have taken place.

The time limits we have set are as follows: A rabbit which survived a milligram for sixty days or one hundredth milligram forty-five days and showed no generalization was considered a human virus. If there was any tendency to generalization with the larger dose, the smaller dose was also used. Practically all bovine viruses were called such on the evidence given by one hundredth milligram doses, many were also tried in one milligram doses, to compare the relative value of the two doses.

To give a complete report of each individual rabbit used is unnecessary. The preceding descriptions give the evidence on which differentiation was made and the findings we considered absolutely typical. In the following descriptions we shall only give the virulence tests which showed some irregularities and discuss the difficulties involved and the methods used to overcome them.

Tabular summaries are also given, which give some idea of the results of the inoculations, time of death, fluctuations in weight, the distribution and severity of the lesions.

In these tables and in the descriptions we have not included the doubtful lesions found in the mammary gland, testicle,

etc. The involvement of the lymph nodes found in rabbits inoculated with human viruses have been included, because of the stress laid upon them. As we have said, confirming the work of Oehlecker to a certain degree, the involvement of the lymph nodes is almost pathognomonic of the bovine type. If, however, as we have found, there are lesions present in the lymph nodes indistinguishable from tuberculous lesions, using the criterion we have adopted, viz., the macroscopic lesion, these then fall into the same position as the spleen for instance. That is, they are involved only occasionally and the findings must be interpreted as part of the whole pathological picture. In a few instances, on the repetition of the virulence tests, a virus was found to have lost its virulence. The results of these inoculations are not used in the tabulations given. In two cases, however, where this loss of virulence was found, the primary tests showed that this virus was less virulent or had already lost some of its virulence. Because of this irregularity in the initial tests, the results are described in the following tables and synopsis:

TESTS ON RABBITS. HUMAN TYPE.

Duration of life according to dose.

	1 Mg.	1/100 Mg.
Died less than 10 days	23	8
" 10 to 20 days	53	24
" 20 to 30 "	61	36
" 30 to 40 "	45	9
" 40 to 50 "	21	9
" 50 to 60 "	7	9
" after 60 days	42	14
Killed after 60 days { Gained weight	175	115
Killed after 60 days { Lost weight	60	34
" 35 to 60 days	6	24
Total	493	282

TESTS ON RABBITS. BOVINE TYPE FROM MAN.

Duration of life according to dose.

	1 Mg.	1/100 Mg.
Died less than 10 days	5	8
" 10 to 20 days	18	3
" 20 to 30 "	13	12
" 30 to 40 "	7	16
" 40 to 50 "	—	13
" 50 to 60 "	—	5
" after 60 days	—	9
Killed after 60 days { Gained weight	—	—
Killed after 60 days { Lost weight	—	2
" 29 to 60 days	—	15
Total	43	83

TESTS ON RABBITS. BOVINE TYPE FROM CATTLE.

Duration of life according to dose.

	1 Mg.	1/100 Mg.
Died less than 10 days	3	4
" 10 to 20 days	6	2
" 20 to 30 "	8	7
" 30 to 40 "	6	16
" 40 to 50 "	1	14
" 50 to 60 "	—	6
" after 60 days	—	15
Killed after 60 days { Gained weight	—	1
Killed after 60 days { Lost weight	—	—
" 35 to 60 days		9
Total	24	74

RABBITS INOCULATED WITH 1 MILLIGRAM OF TUBERCLE BACILLI OF HUMAN TYPE. DISTRIBUTION AND SEVERITY OF LESIONS.

Duration of Life.	Organ Affected.	Number of Total Rabbits Showing Lesions and Relative Severity.					Total for each Organ.		Total Rabbits Inoculated.
		O	+	++	+++	++++	+	−	
15 to 30 days.	Lungs.	18	14	16	26	8	64	18	82
	Kidneys.	58	15	6	3	0	24	58	
	Other lesions.	81	0	1	0	0	1	81	
30 to 45 days.	Lungs.	2	12	15	23	3	53	2	55
	Kidneys.	29	16	7	3	0	26	29	
	Other lesions.	50	5	0	0	0	5	50	
45 to 60 days.	Lungs.	2	8	6	7	1	22	2	24
	Kidneys.	13	8	3	0	0	11	13	
	Other lesions.	24	0	0	0	0	0	24	
60 to 90 days.	Lungs.	10	29	31	10	0	70	10	80
	Kidneys.	27	26	23	4	0	53	27	
	Other lesions.	76	4	0	0	0	4	76	
90 to 120 days.	Lungs.	5	50	55	25	2	132	5	137
	Kidneys.	39	60	32	6	0	98	39	
	Other lesions.	130	7	0	0	0	7	130	
120 to 169 days.	Lungs.	5	17	19	16	3	55	5	60
	Kidneys.	23	24	12	1	0	37	23	
	Other lesions.	56	4	0	0	0	4	56	

RABBITS INOCULATED WITH 1/100 MILLIGRAM OF TUBERCLE BACILLI OF HUMAN TYPE. DISTRIBUTION AND SEVERITY OF LESIONS.

Duration of Life.	Organs Affected.	Number of Total Rabbits Showing Lesions and Relative Severity.					Total for each Organ.		Total Rabbits Inoculated.
		0	+	++	+++	++++	+	−	
20 to 30 days.	Lungs.	16	19	1	0	0	20	16	36
	Kidneys.	27	9	0	0	0	9	27	
	Other lesions.	35	1	0	0	0	1	35	
30 to 45 days.	Lungs.	6	18	5	0	0	23	6	29
	Kidneys.	19	9	1	0	0	10	19	
	Other lesions.	29	0	0	0	0	0	29	
45 to 60 days.	Lungs.	3	9	8	2	0	19	3	22
	Kidneys.	15	3	4	0	0	7	15	
	Other lesions.	22	0	0	0	0	0	22	
60 to 90 days.	Lungs.	8	50	32	8	2	92	8	100
	Kidneys.	47	43	10	0	0	53	47	
	Other lesions.	98	2	0	0	0	2	98	
90 to 120 days.	Lungs.	4	26	18	7	1	52	4	56
	Kidneys.	29	18	8	1	0	27	29	
	Other lesions.	55	1	0	0	0	1	55	
125 to 150 days.	Lungs.	4	1	2	0	0	3	4	7
	Kidneys.	5	1	1	0	0	2	5	
	Other lesions.	7	0	0	0	0	0	7	

RABBITS INOCULATED WITH 1 MILLIGRAM OF TUBERCLE BACILLI OF BOVINE TYPE FROM MAN. DISTRIBUTION AND SEVERITY OF LESIONS.

Duration of Life.	Organs Affected.	Number of Total Rabbits Showing Lesions and Relative Severity.					Total for each Organ.		Total Rabbits Inoculated.
		0	+	++	+++	++++	+	−	
15 to 30 days.	Lungs.	0	0	3	16	8	27	0	27
	Liver.	13	1	4	9	0	14	13	
	Spleen.	6	3	5	13	0	21	6	
	Kidneys.	9	3	13	2	0	18	9	
	Lymph nodes.	14	7	6	0	0	13	14	
	Other lesions.	20	4	3	0	0	7	20	
30 to 45 days.	Lungs.	0	0	0	3	4	7	0	7
	Liver.	3	0	1	3	0	4	3	
	Spleen.	0	0	3	4	0	7	0	
	Kidneys.	1	0	3	3	0	6	1	
	Lymph nodes.	3	1	2	1	0	4	3	
	Other lesions.	4	0	1	2	0	3	4	

RABBITS INOCULATED WITH 1/100 MILLIGRAM OF TUBERCLE BACILLI OF BOVINE TYPE FROM MAN. DISTRIBUTION AND SEVERITY OF CASES.

Duration of Life.	Organs Affected.	Number of Total Rabbits Showing Lesions and Relative Severity.					Total for each Organ.		Total Rabbits Inoculated.
		O	+	++	+++	++++	+	−	
20 to 30 days.	Lungs.	0	0	1	9	3	13	0	13
	Liver.	1	2	3	7	0	12	1	
	Spleen.	0	2	1	8	2	13	0	
	Kidneys.	0	7	4	2	0	13	0	
	Lymph nodes.	5	1	7	0	0	8	5	
	Other lesions.	9	3	1	0	0	4	9	
30 to 45 days.	Lungs.	0	0	1	13	29	43	0	43
	Liver.	9	3	13	18	0	34	9	
	Spleen.	0	1	7	30	5	43	0	
	Kidneys.	0	8	26	9	0	43	0	
	Lymph nodes.	2	7	34	0	0	41	2	
	Other lesions.	18	20	5	0	0	25	18	
45 to 60 days.	Lungs.	0	0	0	0	5	5	0	5
	Liver.	2	1	2	0	0	3	2	
	Spleen.	0	0	1	1	3	5	0	
	Kidneys.	0	1	2	2	0	5	0	
	Lymph nodes.	1	0	4	0	0	4	1	
	Other lesions.	2	1	0	2	0	3	2	
60 to 90 days.	Lungs.	0	0	0	0	6	6	0	6
	Liver.	1	0	1	4	0	5	1	
	Spleen.	1	0	0	2	3	5	1	
	Kidneys.	0	0	2	2	2	6	0	
	Lymph nodes.	0	0	2	4	0	6	0	
	Other lesions.	1	0	3	2	0	5	1	
90 to 98 days.	Lungs.	0	0	0	1	4	5	0	5
	Liver.	4	0	0	1	0	1	4	
	Spleen.	0	0	2	2	1	5	0	
	Kidneys.	0	0	1	3	1	5	0	
	Lymph nodes.	1	1	1	2	0	4	1	
	Other lesions.	1	0	2	1	1	4	1	

RABBITS INOCULATED WITH 1 MILLIGRAM OF TUBERCLE BACILLI OF BOVINE TYPE FROM CATTLE. DISTRIBUTION AND SEVERITY OF LESIONS.

Duration of Life.	Organs Affected.	Number of Total Rabbits Showing Lesions and Relative Severity.					Total for each Organ.		Total Rabbits Inoculated.
		0	+	++	+++	++++	+	−	
15 to 30 days.	Lungs.	0	0	2	5	6	13	0	13
	Liver.	5	2	2	4	0	8	5	
	Spleen.	4	1	3	4	1	9	4	
	Kidneys.	3	5	5	0	0	10	3	
	Lymph nodes.	7	4	2	0	0	6	7	
	Other lesions.	10	2	1	0	0	3	10	
30 to 45 days.	Lungs.	0	0	0	1	5	6	0	6
	Liver.	1	0	3	2	0	5	1	
	Spleen.	0	1	3	2	0	6	0	
	Kidneys.	1	3	2	0	0	5	1	
	Lymph nodes.	0	0	5	1	0	6	0	
	Other lesions.	2	1	2	1	0	4	2	
45 to 60 (47 days).	Lungs.	0	0	0	0	1	1	0	1
	Liver.	1	0	0	0	0	0	1	
	Spleen.	1	0	0	0	0	0	1	
	Kidneys.	0	0	0	1	0	1	0	
	Lymph nodes.	0	0	1	0	0	1	0	
	Other lesions.	0	0	0	1	0	1	0	

RABBITS INOCULATED WITH 1/100 MILLIGRAM OF TUBERCLE BACILLI OF BOVINE TYPE FROM CATTLE. DISTRIBUTION AND SEVERITY OF LESIONS.

Duration of Life.	Organs Affected.	Number of Total Rabbits Showing Lesions and Relative Severity.					Total for each Organ.		Total Rabbits Inoculated.
		O	+	++	+++	++++	+	−	
20 to 30 days.	Lungs.	0	0	0	5	2	7	0	7
	Liver.	0	0	5	2	0	7	0	
	Spleen.	0	0	3	4	0	7	0	
	Kidneys.	0	4	3	0	0	7	0	
	Lymph nodes.	1	1	5	0	0	6	1	
	Other lesions.	3	2	2	0	0	4	3	
30 to 45 days.	Lungs.	0	0	0	6	27	33	0	33
	Liver.	3	2	11	17	0	30	3	
	Spleen.	0	0	3	25	5	33	0	
	Kidneys.	0	2	29	2	0	33	0	
	Lymph nodes.	2	3	28	0	0	31	2	
	Other lesions.	15	8	6	4	0	18	15	
45 to 60 days.	Lungs.	0	0	0	0	12	12	0	12
	Liver.	1	2	8	1	0	11	1	
	Spleen.	0	0	2	8	2	12	0	
	Kidneys.	0	2	8	2	0	12	0	
	Lymph nodes	1	0	11	0	0	11	1	
	Other lesions.	5	5	1	1	0	7	5	
60 to 90 days.	Lungs.	0	0	0	1	12	13	0	13
	Liver.	3	0	6	4	0	10	3	
	Spleen.	0	0	5	3	5	13	0	
	Kidneys.	0	0	8	3	2	13	0	
	Lymph nodes.	1	0	9	3	0	12	1	
	Other lesions.	5	2	1	4	1	8	5	
90 to 161 days. (One rabbit only over 100 days.)	Lungs.	0	0	0	1	2	3	0	3
	Liver.	0	0	2	1	0	3	0	
	Spleen.	0	0	1	2	0	3	0	
	Kidneys.	0	0	2	1	0	3	0	
	Lymph nodes.	1	0	2	0	0	2	1	
	Other lesions.	0	0	0	0	0	0	0	

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS.

From Man.

Diagnosis of Case.	No.	Dose.	Autopsy.
Pulmonary tuberculosis. Adults	13. II. type.	1 mg.	Killed 116 days. Gain 175 grams. Lungs uniformly studded with tubercles. One small tubercle in each kidney, spleen somewhat enlarged and contains a few tubercles.
" " "	" "	.01 mg.	Killed 90 days. Gain 280 grams. Lungs, very few small tubercles. Kidneys contain a few tubercles.
" " "	22. II. type.	1 mg.	Died 128 days. Loss 20 grams. Enteritis. Lungs, 10–20 tubercles. One enlarged inguinal node, size, lima bean, described as caseous. Tuberculous?
" " "	27. II. type.	1 mg.	Killed 114 days. Gain 430 grams. Lungs well involved. Spleen is slightly enlarged, shows a few tubercles.
" " "	" "	.01 mg.	Killed 99 days. Gain 770 grams. No tuberculous lesions.
" " "	36. II. type.	1 mg.	Died 30 days. Loss 515 grams. Lungs semi-collapsed and studded with tubercles. Kidneys, many pin-point tubercles. Spleen, not enlarged, many small tubercles.
" " "	" "	.01 mg.	Killed 134 days. Gain 150 grams. Lungs, tubercles chiefly at edges of lobes. Kidneys, 12–15 medium-sized tubercles. Large subcutaneous abscess on abdomen, with enlargement of axillary node (result of bite).
" " "	" "	1 mg.	Died 83 days Loss 20 grams. Lungs, a great many confluent tubercles. Kidneys, many large tubercles.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS. — *Continued.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Pulmonary tuberculosis. Adults, *cont.* . . .	105. H. type.	1 mg.	Died 125 days. Loss 10 grams. Had lost weight at first and then gained. Lungs, many fine tubercles. Kidneys, few tubercles, (tuberculous?) orchitis. Peritoneum, marked tuberculosis.
" " "	" "	.01 mg.	Killed 105 days. Gain 505 grams. Lungs, 4–5 large confluent tuberculous areas. Kidneys, one or two small tubercles.
" " "	118. H. type.	1 mg.	Died 40 days. Loss 500 grams. Few tubercles in lungs. Otherwise negative.
" " "	" "	.01 mg.	Died 85 days. Loss 373 grams. Lungs, few caseous tubercles of moderate size. Kidneys, 4–5 medium-sized tubercles. Axillary and inguinal nodes show tubercles. Coccidiosis.
" " "	232. H. type.	1 mg.	Killed 119 days. Gain 180 grams. Lungs, partially collapsed; moderate number of medium sized tubercles; several very large confluent areas. Kidneys, very few small tubercles, one large one. Lymph node at tarsal region, enlarged, caseous appearance (tuberculous?).
" " "	366. H. type.	1 mg.	Killed 85 days. Lost weight at first, then gained. Total loss 85 grams. Lungs, very few small tubercles. Inguinal node, on one side, shows apparent tubercles and is caseous in its center.
" " "	415. H. type.	1 mg.	Died 38 days. Loss 445 grams. Axillary and inguinal nodes enlarged, possibly small tubercles. Kidneys, numerous tubercles. Lungs, partially collapsed and studded with tubercles. Solid caseous areas at margins of lower lobes.

"	"	"	"	"	.01 mg.	Died 61 days. Loss 225 grams. Many small tubercles in lungs. Rabbit septicemia.
"	"	"	419.	H. type.	1 mg.	Died 31 days. Loss 575 grams. Axillary nodes, much enlarged, congested, contain small tubercles. Kidneys, few very small tubercles. Lungs, collapsed, small areas of consolidation, with very small tubercles in moderate numbers.
"	"	"	"	"	.01 mg.	Killed 87 days. Gain 230 grams. Lungs, very few scattered tubercles. Kidneys, many small tubercles.
"	"	"	459.	H. type.	1 mg.	Killed 90 days. Gain 340 grams. Lungs, rather numerous tubercles, majority small, some confluent. Kidneys, one large tubercle in each. Few tubercles in large intestine, in what are apparently lymph follicles. No ulceration.
"	"	"	485.	H. type.	.01 mg.	Killed 87 days. Loss 70 grams. Lungs, a few small tubercles. Kidneys, one tubercle. Large intestine, a few tubercles.
Tuberculous adenitis.	Adults		646.	H. type.	1 mg.	Died 19 days. Loss 375 grams. Lungs and kidneys show a few small tubercles.
"	"	"	"	"	.01 mg.	Killed 93 days. Gain 285 grams. Lungs, partially collapsed. Many confluent tubercles, especially on posterior surface. Right kidney, one tubercle. Spleen, two small tubercles.
Tuberculous adenitis.	Children		184.	B. type.	1 mg.	Died 17 days. Loss 485 grams. Lungs do not collapse, riddled with small tubercles. Possible tubercles in superficial nodes. Kidneys, few tubercles. Spleen enlarged, no tubercles.
"	"	"	"	"	.01 mg.	Died 38 days. Loss 795 grams. Complete generalization.
"	"	"	"	"	.01 mg.	Died 33 days. Loss 300 grams. Complete generalization.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS. — *Continued.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Tuberculous adenitis. Children, *cont.* ...	217. B. type.	1 mg.	Died 18 days. Loss 365 grams. Axillary nodes, pin-point tubercles. Lungs, many small tubercles. Spleen enlarged, no tubercles.
" " "	" "	1 mg.	Died 20 days. Loss 365 grams. Generalization more marked.
" " "	" "	1 mg.	Died 20 days. Loss 610 grams. Generalization more marked.
" " "	" "	1 mg.	Died 20 days. Loss 500 grams. Generalization more marked.
" " "	" "	.01 mg.	Died 29 days. Loss 525 grams. Generalization complete.
" " "	272. H. type.	1 mg.	Killed 122 days. Loss 740 grams. Right radius and left carpal joint involved, considered tuberculous. Lungs, many confluent caseous tubercles. Kidneys, many tubercles. Costal cartilages, two at sternal and involved, considered tuberculous.
" " "	272. H. type.	1/100 mg.	Died 27 days. Loss 255 grams. Axillary node, tubercles(?). Lungs, small tubercles scattered throughout. Kidneys, few small tubercles.
" " "	" "	1 mg.	Died 33 days. Loss 810 grams. Lungs, partially collapsed; many small tubercles. Kidneys, one or two tubercles. Enteritis.
" " "	" "	.01 mg.	Killed 93 days. Gain 520 grams. Lungs, very few large caseous tubercles. Kidneys, one tubercle.
" " "	381. B. type.	1 mg.	Died 15 days. Loss 576 grams. Hardly visible tubercles in lungs.
" " "	" "	.01 mg.	Died 27 days. Loss 555 grams. Complete generalization.

" " "	396.	H. type.	1 mg.	Killed 91 days. Gain or loss 0. Lungs, few tubercles. Axillary and inguinal nodes markedly involved with apparently caseous areas. Considered tuberculous.
Abdominal tuberculosis. Children	109.	B. type.	1 mg.	Died 19 days. Loss 435 grams. Lungs do not collapse, peppered with small tubercles. Kidneys, many small tubercles. Spleen, no tubercles. Liver, very fine tubercles. Mass of caseous-like material at sacculus rotundus.
" " "	"	"	.01 mg.	Died 84 days. Loss 140 grams. Very marked lesions. Spleen and liver not involved.
" " "	"	"	.01 mg.	Died 30 days. Loss 770 grams. Complete generalization.
" " "	"	"	.01 mg.	Died 36 days. Loss 430 grams. Complete generalization.
" " "	"	"	.01 mg.	Died 29 days. Loss 105 grams. Complete generalization.
" " "	"	"	.01 mg.	Killed 29 days. Loss 70 grams. Complete generalization.
" " "	604.	H. type.	1 mg.	Killed 88 days. Gain 10 grams. Lungs, moderate number of rather small tubercles. Kidneys, moderate number of medium tubercles. Spleen, few possible tubercles. Inguinal node involved, appearance caseous (tuberculous?).
" " "	"	"	.01 mg.	Killed 88 days. Gain 220 grams. Lungs, a moderate number of confluent tubercles. Kidneys, one small tubercle in each.
Generalized tuberculosis. Children	99.	H. type.	1 mg.	Killed 152 days. Gain 360 grams. Lungs entirely collapsed, few tubercles. Spleen, two tubercles, size of a pin head. No other tuberculous lesions.
" " "	"	"	.01 mg.	Died 21 days. Loss 200 grams. Lungs, few tubercles. Kidneys, two or three tubercles. Enteritis.
				Note. — Other strains from this case typical in rabbits.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS. — *Continued.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Generalized tuberculosis. Children, *cont.*,	122. B. type.	1 mg.	Died 16 days. Loss 810 grams. Lungs, many tubercles. Kidneys, few tubercles.
" " "	" "	.01 mg.	Died 32 days. Loss 355 grams. Generalization complete.
" " "	" "	.01 mg.	Died 40 days. Loss 520 grams. Generalization complete.
" " "	123. B. type. (See 122.)	1 mg.	Died 16 days. Loss 80 grams. Lungs and kidneys, moderate tuberculosis.
" " "	123. B. type.	.01 mg.	Died 38 days. Loss 400 grams. Complete generalization.
" " "	" "	.01 mg.	Died 48 days. Loss 415 grams. Complete generalization.
" " "	274. H. type.	1 mg.	Killed 118 days. Gain 80 grams. Lungs, collapsed, a few scattered tubercles. Kidneys, numerous, large tubercles. Axillary node, tubercles.
" " "	689. H. type.	1 mg.	Killed 85 days. Loss 330 grams. Lungs, moderate number of small and larger caseous tubercles, scattered. Kidneys, numerous small tubercles. Intestine, few tubercles in large and small portions.
" " "	692. H. type. (See 689.)	1 mg.	Died 28 days. Loss 495 grams. Lungs, moderate number of very small tubercles. Kidneys, very many small tubercles. Axillary and inguinal nodes, a few very small tubercles. Spleen, numerous small tubercles.
" " "	692. H. type.	.01 mg.	Died 78 days. Loss 430 grams. Lungs, moderate number of medium, confluent tubercles. No other tuberculous lesions. Enteritis.

" " "	" "	1 mg.	Died 25 days. Loss 660 grams. Lungs do not collapse; riddled with many fine tubercles. Kidneys, moderate number of small tubercles. One suspicious area in one inguinal and one axillary node. Coccidiosis.
" " "	" "	1 mg.	Killed 95 days. Loss 180 grams. Lungs collapsed, many small and medium-sized tubercles, some with small caseous centers. Kidneys, many large and medium-sized caseous tubercles. Coccidiosis.
" " "	"	.01 mg.	Died 24 days. Loss 340 grams. Lungs, few scattered tubercles. Septicemia.
Tuberculosis of bones and joints	586. H. type.	1 mg.	Killed 93 days. Loss 465 grams. Lungs, a few confluent tubercles in lower lobes. Kidneys, rather numerous tubercles of varying size. Large intestine, few tubercles in the mucosa. Abscess of mammary gland.
" " " "	" "	.01 mg.	Died 88 days. Loss 770 grams. Lungs, very few medium-sized tubercles. One large caseous tubercle in lower lobe. Septicemia.
" " " "	662. H. type.	1 mg.	Died 16 days. Loss 415 grams. No tuberculosis. Enteritis.
" " " "	" "	.01 mg.	Died 57 days. Loss 435 grams. Lungs, moderate number of medium, caseous tubercles; two or three very large ones. Kidneys, negative. Coccidiosis and septicemia.
" " " "	" "	1 mg.	Died 37 days. Loss 1,155 grams. Lungs do not collapse; very small and larger tubercles. Axillary and mesenteric nodes, many medium-sized tubercles. Spleen, one possible tubercle. Liver, coccidiosis.
Genito-urinary tuberculosis.....	621. H. type.	1 mg.	Died 21 days. Loss 285 grams. Lungs, very few tubercles. Coccidiosis.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS.— *Continued.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Genito-urinary tuberculosis, *cont.*	621. H. type.	1 mg.	Died 20 days. Loss 340 grams. Numerous small tubercles throughout lungs.
" "	" "	1 mg.	Died 37 days. Loss 810 grams. Lungs, many small tubercles. Inguinal nodes, right, small tubercles. Kidneys, moderate number of tubercles. Coccidiosis.
" "	" "	.01 mg.	Killed 94 days. Loss 120 grams. Lungs, few small tubercles. No other tuberculous lesions.
" "	649b. H. type.	1 mg.	Died 28 days. Loss 610 grams. Lungs, few tubercles.
" "	" "	1 mg.	Killed 84 days. Gain 30 grams. Lungs, scattered tubercles, moderate number of medium size with caseous centers. Kidneys, many tubercles of medium and large size. Spleen, two small tubercles. Liver, possible tubercles; about six suspicious areas. No frank coccidiosis.
" "	" "	1/100 mg.	Killed 84 days. Gain 150 grams. No tuberculosis.
Bovine Viruses.			
Milk	89	.01 mg.	Died 85 days. Loss 110 grams. Lungs, large confluent tubercles, showing caseation. Bronchial nodes, tuberculous. Kidneys and spleen show few large tubercles. This strain was overlooked and above test was late, that is on the ninth generation. Repetitions were made because of apparent low virulence shown by amount of lesions and length of life.

"	89	.01 mg.	Killed 35 days. Gain 70 grams. Lungs, moderate number of medium and large tubercles with caseous centers. Kidneys, one tubercle.
"	89	.01 mg.	Killed 35 days. Gain 40 grams. Lungs, a few scattered tubercles.
"	89	.01 mg.	Killed 36 days. Loss 70 grams. Lungs, moderate number of small tubercles. Kidneys, few tubercles. Spleen, 3–4 small tubercles.
"	89	.01 mg.	Killed 36 days. Gain 390 grams. Lungs, a very few small or larger tubercles. Kidneys, one tubercle.
"	89	.01 mg.	Killed 105 days. Original weight. Lungs, few scattered tubercles. Kidneys, few tubercles.
"	89	.01 mg.	Killed 105 days. Gain 45 grams. Lungs, few caseous tubercles.
"	89	.01 mg.	Killed 105 days. Gain 505 grams. No tuberculosis.
"	89	.01 mg.	Killed 105 days. Gain 140 grams. Few tubercles in lungs and kidneys.
"	89	1 mg.	Killed 105 days. Gain 340 grams. Lungs, a few scattered tubercles. Kidneys, few tubercles.
"	89	1 mg.	Killed 105 days. Loss 35 grams. Lungs, do not collapse, filled with large caseous and smaller tubercles. Liver and spleen, a few possible tubercles. Kidneys, many tubercles. Mediastinal nodes tuberculous.

This series of tests showed that the strain had almost completely lost its virulence. A duplicate strain which had been dropped was then brought into cultivation and its virulence tested.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS. — *Continued.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Milk, *cont.*	89a	1 mg.	Died 23 days. Loss 410 grams. Lungs, do not collapse, many small tubercles. Spleen and liver, many small tubercles. Kidneys, a few small tubercles.
"	89a	.01 mg.	Killed 35 days. Loss 175 grams. Lungs, moderate number of small tubercles. Liver, many small tubercles. Spleen, filled with medium-sized tubercles. Kidneys, several small tubercles.
"	89a	.01 mg.	Killed 62 days. Gain 30 grams. Lungs, do not collapse; many confluent caseous tubercles. Heart, one large tubercle Spleen, many small tubercles. Kidneys, many large tubercles. Omentum, rolled up and tubercular. Liver, few tubercles. Superficial lymph nodes, few small tubercles. The duplicate strain has retained its power of generalization, but does not kill the animal quickly, nor cause it to fail rapidly.
Cattle	271	.01 mg.	Killed 161 days. Gain 490 grams. Lungs, semi-collapsed, numerous medium-sized confluent caseous tubercles. Kidneys, few small tubercles; one is .5 centimeter in diameter and caseous. Liver and spleen, few small tubercles (culture, sixth generation). Although the dose was weighed for this rabbit, comparison with the succeeding three makes it very probable that with the small amount of growth available the dose was too small. This seems the most reasonable explanation of the long time of survival. Other than this the strain is fully virulent.

		1 mg.	Died 31 days. Loss 705 grams. Axillary and inguinal nodes show small tubercles. Lungs solid with innumerable medium-sized caseous tubercles. Peritoneum, scattered tubercles. Spleen very much enlarged, only a few tubercles. Kidneys and liver, no tubercles.
"	271	.01 mg.	Killed 35 days. Loss 470 grams. Lungs voluminous, riddled with confluent tubercles with caseous centers. Liver, very many small and pin-point tubercles. Spleen much enlarged, closely studded with medium-sized tubercles. Kidneys, moderate number of tubercles. Bronchial and iliac nodes, few tubercles.
"	271	.01 mg.	Died 64 days. Loss 570 grams. As above, with tubercles over peritoneum.
"	311	1 mg.	Died 21 days. Loss 540 grams. Lungs do not collapse, many tubercles. Kidneys, few tubercles.
"	311	.01 mg.	Died 36 days. Loss 280 grams. Complete generalization.
"	314	1 mg.	Died 47 days. Loss 875 grams. Lungs do not collapse, very many confluent tubercles. Kidneys, numerous tubercles. Liver and spleen negative. Axillary and inguinal nodes, tubercles. Peritoneum, many pin-point tubercles. The dose for this rabbit was only estimated. The length of life is greater than that of any other rabbit receiving this dose. The generalization is incomplete. The degree of involvement is, however, severe.
			The following two rabbits were inoculated at the same time.
"	314	.01 mg.	Killed 53 days. Loss 248 grams. Lungs riddled with confluent caseous tubercles. Bronchial node caseous. Liver, tubercles? Coccidiosis. Spleen very large, shot with confluent caseous tubercles. Kidneys, moderate number of large tubercles. Peritoneum, tubercles over parietal and visceral layer. Heart, one tubercle. Axillary and inguinal nodes enlarged, confluent caseous tubercles.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS. — *Continued.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Cattle, *cont.*	314	.01 mg.	Died 66 days. Loss 115 grams. Lungs riddled with confluent caseous tubercles. Tubercles over diaphragm and heart. Kidneys, numerous tubercles. Spleen, enormously enlarged, large caseous tubercles. Liver, tubercles, but coccidia present. Superficial and retroperitoneal nodes enlarged; confluent caseous tubercles. This series of rabbits shows in an extreme manner the advantages of the .01 milligram dose. Even with only an added 6-day interval the lesions are much more widespread. (As this virus was irregular culturally many virulence tests were made both in the original cultures and after passage through guinea-pigs or rabbits. These all gave typical results, except one rabbit dying in 18 days, dose 1 milligram, where the spleen escaped, otherwise generalization was marked. Subcutaneous inoculations of 5 to 10 milligrams gave the same results.)
"	330	.01 mg.	Died 121 days. Gain 15 grams. Lungs solid and riddled with large confluent caseous tubercles. Kidneys, moderate number of large caseous tubercles. Spleen, enlarged, moderate number of large caseous tubercles. Liver, few fine tubercles. Tubercles in rib marrow. Peritoneum, few tubercles. Axillary bronchial and retroperitoneal nodes contain large caseous tubercles. (Culture generation 4th, weighed amount.) As this length of life and the slight change in weight was exceptional repetitions were made.

"	330	1 mg.	Died 49 days. Loss 745 grams. Moderate involvement of lungs and kidneys. Very doubtful tubercles in liver and spleen. Coccidiosis.
"	330	.01 mg.	Died 115 days. Loss 120 grams. Lungs, very marked lesions. Kidneys, few tubercles. Spleen enlarged, one tubercle. Liver, negative.
			(The two preceding tests on the 7th generation.)
"	330	.01 mg.	Killed 35 days. Loss 20 grams. Moderate tuberculosis of lungs and kidneys.
"	330	1 mg.	Died 28 days. Loss 655 grams. Lungs, many discrete tubercles. Spleen, many small discrete tubercles. Kidney, few tubercles. Small tubercle in one inguinal node.
"	330	.01 mg.	Killed 89 days. Gain 435 grams. Few scattered tubercles in the lungs.
			(The three preceding tests on the 11th generation.)
			This virus quickly declined in virulence, and had already lost virulence or was below the average by the fourth generation.
"	334	1 mg.	Died 18 days. Loss 310 grams. Lungs do not collapse, many tubercles.
"	334	1 mg.	Died 26 days. Loss 910 grams. Lungs riddled with tubercles. Kidneys, few tubercles.
"	334	.01 mg.	Killed 48 days. Loss (not noted). Complete generalization.
"	334	.01 mg.	Died 34 days. Loss 540 grams. Complete generalization.

VIRUSES SHOWING VARIATIONS IN THEIR EFFECT ON RABBITS. — *Concluded.*

Diagnosis of Case.	No.	Dose.	Autopsy.
Cattle, *cont.*	334	.01 mg.	Died 39 days. Loss 385 grams. Complete generalization.
"	334	.01 mg.	Killed 35 days. Loss 390 grams. Complete generalization.
"	334	.01 mg.	Died 43 days. Loss 390 grams. Complete generalization.
"	334	.01 mg.	Died 53 days. Loss 410 grams. Complete generalization.
"	334	.01 mg.	Died 53 days. Loss 235 grams. Complete generalization.
"	493	.01 mg.	Killed 35 days. Gain 80 grams. Lungs and kidneys only involved. Culture generation 8.
			(This was a retest made to find the time of generalization. The virus gave typical results in every other inoculation, except that the length of life was longer than the average, as was also shown by the rabbit inoculated at the same time as the above.)

The preceding tables and descriptions show that the involvement of organs other than the lungs and kidneys is unusual in rabbits receiving a human virus. It also shows that this was present in only four rabbits receiving .01 milligram, but was present in twenty-one receiving one milligram. Of the first four only one showed involvement of the spleen, of the latter twenty-one, six showed involvement of the spleen and two doubtful involvement. Another of this series showed tuberculous peritonitis. The few rabbits showing involvement of the intestine we do not consider as significant. The involvement of the lymph nodes in two rabbits receiving .01 milligram and in eleven rabbits receiving one milligram, some of which lesions are doubtful, has been spoken of.

This shows that in one milligram doses the human virus has in a very small number of the cases a slight capacity to spread. With .01 milligram this capacity is shown in a still less degree. If one compares the results in a series of rabbits one finds that this is not constant. That some human viruses are more virulent than others is admitted, but any scale of virulence based on such findings is impossible as rabbits in a series vary so much. For this reason we consider these occasional variations, or tendency to include more organs than the type to be chance variations in individual animals.

When we consider the variations in the bovine viruses there is the same variation with the larger dose and practically none with the smaller dose. The main variation in the one milligram dose has been failure to generalize. When this occurred between the fifteenth and twentieth days one could consider the time too short by analogy with later autopsies. The fact that generalization may occur as soon as this in some rabbits is no objection to the conclusion that the time is too short to expect generalization in every rabbit used. Of the rabbits dying later, one at twenty-six days failed to show generalization and one lived the exceptional time of forty-seven days with lesions widespread but with escape of liver and spleen. Every rabbit, however, inoculated with .01 milligram of these same viruses showed typical generalization, and the only variation was in one rabbit where the

spleen and liver escaped, although the lesions were otherwise widespread.

Why it is that the smaller dose gives so much wider generalization and more marked and striking picture in instances where the period of time is only slightly longer we cannot say. It is possible that the body when overwhelmed does not react with macroscopic tissue changes, the disease in this case being multiplication of the bacteria with intoxication and little response from the infected animal. In the majority of cases the distinctive picture is wholly dependent on retardation of the disease and the subsequently larger and more striking lesions.

In one instance a rabbit receiving .01 milligram gained in weight and was killed after one hundred and sixty-one days, and though the lesions were generalized they were not severe. Control rabbits made it evident that this rabbit probably received a much smaller dose than was thought.

In another instance of two rabbits injected with .01 milligram one failed to show generalization and the other died in eighty-nine days and showed generalization. This test was with the eighth generation. Test made in the second generation gave fatal generalization in forty-nine days. Though it was not among the most virulent at the start the retests showed some diminution.

The rabbit results are given on two viruses which showed more marked loss of virulence. One not tested till the ninth generation had either lost some degree of virulence or was less virulent than the average. The test was repeated and by this time the greater part of the virulence was lost. The duplicate strain, though isolated at the same time, had not been in active cultivation but stored in the cold. When a fresh transfer was inoculated it was able to cause fatal generalized tuberculosis. As in the cultural work, this virus showed variation in two strains of the same virus. It is possible that the degree of active cultivation may have influenced the results. Another virus only killed the rabbit after one hundred and twenty-one days, cultures in the fourth generation being used. Retests gave irregular results, but

virulence was lost to a great degree. The uniform generalization in each rabbit was absent. One other strain not tabulated, as it was very virulent at the start, has almost completely lost its virulence.

The above findings show the importance of using early generations for measuring the virulence of a strain. Although our work as well as that of others has shown that there is a marked degree of stability in this characteristic still, where one is using this characteristic for differentiation, no reliance should be put upon such stability in individual cultures.

The virulence tests with the following viruses are described for the same reason that we gave their cultural characteristics — because they are unusual in origin. Otherwise they conform in every way to the type to which we have assigned them.

TABLE.

Diagnosis of Case.	No.	Dose.	Autopsy.
Generalized tuberculosis. Children.....	634. B. type.	1 mg.	Died 11 days.
" " "	" "	.01 mg.	Killed 31 days. Loss 155 grams. Lungs partially collapsed, very many confluent tubercles, with small caseous centers. Axillary, inguinal and retroperitoneal nodes, small tubercles. Liver, many fine tubercles throughout. Spleen, many small tubercles. Kidneys, a few tubercles.
" " "	" "	.01 mg.	Died 40 days. Loss 770 grams. Axillary and inguinal nodes, doubtful tubercles. Lungs, upper lobes do not collapse, many large confluent tubercles throughout. Liver, many fine tubercles. Spleen, a few small tubercles. Kidneys, same.
" " "	651. H. type. (See 634.)	1 mg.	Killed 86 days. Gain 570 grams. Lungs, a few moderate-sized tubercles. Kidneys, moderate number of small tubercles.
" " "	" "	.01 mg.	Died 21 days. Loss 355 grams. Lungs, very few tubercles.
Genito-urinary tuberculosis.....	199. B. type.	1 mg.	Died 21 days. Loss 395 grams. Lungs do not collapse, riddled with small tubercles. Heart, 10–12 small tubercles. Spleen, much enlarged, few small tubercles. Kidneys, moderate number of small tubercles. Possible tubercles in inguinal nodes.
" "	" "	.01 mg.	Died 38 days. Loss 705 grams. Axillary and inguinal nodes enlarged, small caseous tubercles. Lungs, mass of confluent tubercles. Spleen riddled with small tubercles. Kidney, moderate number of tubercles.

" "	" "	.01 mg.	Died 38 days. Loss 425 grams. Axillary and inguinal nodes enlarged; confluent, caseous tubercles. Lungs, solid mass of confluent tubercles. Spleen, enlarged and riddled with tubercles. Many tubercles on peritoneum and in kidneys.
Viruses from milk.....	III. H. type.	1 mg.	Killed 80 days. Gain 550 grams. Entirely negative except for 1–2 tubercles in each kidney and a few in the lungs, which are well collapsed.
" " "	IIIa. H. type.	1 mg.	Killed 113 days. Well nourished. Large axillary abscess. Kidneys, few small tubercles. Lungs, collapsed for most part; few tubercles of moderate size.

The conclusions again briefly stated are as follows: The rabbit is the best animal to use for testing virulence for diagnosis of type. The intravenous inoculation of .01 milligram of culture is the best method for eliciting these differences. Any virus that is capable of causing progressive, generalized tuberculosis with this dose is of the bovine type, the extent of the lesions being most important, the time of death being less important. All viruses incapable of causing lesions elsewhere than in the lungs or kidneys or both are of the human type. Chance localization elsewhere is only occasional and not constant and is ruled out because a bovine virus is capable of causing generalization in every rabbit inoculated. It is essential that all virulence tests be made with early generations.

Correlation of results. — As is evident from the foregoing, our results are a confirmation of those of Oehlecker. Comparing them with others who have used the intravenous route we find practical agreement. Where there is disagreement this is only apparent, being due to the size of the dose. Deductions from one milligram or two milligram doses are to be made with caution. Those of Rabinowitsch and Fibiger and Jensen are unwarranted as is evident from the description of our own results.

When we compare our results with those using different methods of inoculation, there is little to say further than that there is substantial agreement. Kossel, Weber and Heuss find the same differences in virulence using the subcutaneous method, and the delicacy of the rabbit test is shown in their case of mixed infection where culture inoculations into rabbits solved the question. The results of the Royal Commission are practically the same. They used different methods of inoculation, especially the intraperitoneal route. The results of some of their experiments show the striking susceptibility of the rabbit to the bovine virus, thus, "even very minute doses, such as one hundred-thousandth or one millionth of a milligram injected either intraperitoneally or

subcutaneously surely produce progressive tuberculosis in this animal."

In the few instances where apparent irregularities are reported, as in the use of the subcutaneous method, there has usually been some more extensive involvement in individual rabbits than commonly found and usually of the lungs or kidneys. Even in these cases the length of life or comparison with other rabbits inoculated from the same material leaves little doubt that a human virus was being used, especially when one compares the findings with the results of inoculation of bovine viruses made by the same worker. In a very few instances the data are insufficient and above all, the possibility of a mixed infection is not excluded. Where the work is incomplete, naturally, the case is of no value as evidence of irregularity.

Taking all facts into consideration and carefully studying the protocols of different workers, the advisability of using small doses becomes very evident. As is shown in our work the small dose leads to a more slowly progressing disease, which gives the greatest anatomical contrast between the two types. Whether the reduction of the dose with other methods of inoculations would cause the few dissenters to class their viruses as typical we cannot say, but it is likely that the so-called atypical results would be few. Although the majority of rabbits will stand very large doses of human viruses, occasionally a strain shows greater virulence or the individual rabbits show variations. With the minimum dose which, with bovine viruses would always cause generalized tuberculosis in rabbits, we think that the contrast would be at its maximum.

Although we have had little experience with the different methods of inoculation, from careful comparison of results we find the intravenous method in every way satisfactory. Although we adopted this method thinking from the descriptions already published that it would be the quickest method, the necessary reduction of the dosage has rendered this factor negligible. Although the gross pathological contrast is not better than with the subcutaneous method, still the

absence of large abscesses which may open seems an advantage, and further, one knows absolutely where the injection is going. The intraperitoneal method appears to be the least advantageous, due to the lessened anatomical contrast.

VIRULENCE FOR CALVES.

Resumé. — There are probably no more discordant results to be found in any branch of bacteriology than in the early experiments on the virulence of tubercle bacilli of human and bovine sources for cattle. The essential reason for this lack of agreement is the complete absence of correlation and uniformity of experimentation. Much of the work must be excluded from consideration for this reason and especially in the light of the uniform results where coördinate and comparable methods are used.

Smith in his first article called attention to the differences in virulence in calves inoculated under the same conditions. The bovine culture caused progressive tuberculosis and death, whereas the culture (*Nasua narica*), presumably human, caused no tuberculosis.

Koch's announcement in 1901, and the reports of the work of Koch and Schütz, who were unable to cause progressive lesions by inoculation of tubercle bacilli from man either with cultures or sputum, whereas bovine tubercle bacilli caused generalized tuberculosis, called forth a large amount of experimental evidence.

In considering the work that immediately followed Koch's announcement some conclusions can be drawn if the sources of the discordant results and conclusions are considered.

The possibility that tuberculous lesions could be produced experimentally although cattle might be completely immune under natural conditions was lost sight of. This is evident in the dosage and methods used. The inoculations were either intravenous, intrathoracic, intraperitoneal, or subcutaneous, and in some instances two or more points were used in the same animal. In practically all of these experiments sputum with its numerous contaminations, tissue emulsions from man direct or from inoculated guinea-pig

organs were used. The dose, therefore, must have fluctuated within wide limits. Under these conditions greatly varying results were obtained which were further obscured by the individual interpretations of what constituted a successful transference to cattle.

The feeding and inhalation experiments were more uniform, but the lesions found were also variously interpreted.

It is possible that in some instances experiments were done with a bovine virus from man though one is unable to pick out these cases, due to the great irregularity of the results as a whole. Another source of error, spontaneous tuberculosis in the cattle used, was not always rigidly excluded.

Much of the controversy founded on these wholly uncorrelated experiments seems to us now beyond the point. Whatever interpretation we place on the lesions produced, whether true tuberculosis or due to a foreign toxic body, makes little difference. The sum total of this work only proved that the bovine virus was far more virulent for cattle and that the human virus showed little power to cause progressive lesions.* The more rational method of comparing the virulence of the two viruses in measured doses and the examination of cultures from cattle for the possible occurrence of a virus corresponding to the human type has given us far more information as to the possibility of infection of cattle from human sources.

In the work of determining the existences of two types of bacilli, the virulence for cattle was a crucial point. If a virus from man was of the bovine type it would have to be shown to be virulent for its natural host.

Besides the pioneer work of Smith, the comparative and careful work of Kossel, Weber and Heuss and later that of the Royal Commission are especially important in the determination of the differences in virulence and the value of this difference for the determination of type.

The workers of the *Gesundheitsamt* have had practically

* For complete summary see Weber, Die Tuberkulose des Menschen und der Tiere, Kolle and Wasserman, Erster Ergänzungsband, p. 119.

uniform results. All human viruses without exception have failed to show any power of generalization, whereas the bovine viruses from man or animals have shown this power. In the latter case an acutely fatal or a slowly progressive non-fatal disease was produced. In one instance one calf in a series was refractory. The authors think that there was an immunity established in this calf due to a healed tuberculosis. The method used was the subcutaneous inoculation of fifty milligrams of culture. They show the advantage of the intravenous method as a control in doubtful cases and also point out its possible fallacies. Thus certain bacilli of the human type are so highly toxic that even five milligrams in calves cause marked illness or even death. If this occur acutely, tuberculous lesions will be found in the lungs, but if they survive four months and are killed, no lesions are present. Tuberculin made from one of these strains killed a tuberculous guinea-pig when used in the extremely low dose of .02 cubic centimeter.

They point out how great a source of error spontaneous tuberculosis may be. In their work such errors amounted to nine per cent. It was found necessary to narrow the question of a negative tuberculin reaction very much. How serious this may be is shown by their figures. Thus, in one instance, of a shipment of fifteen calves, six or forty per cent reacted, the remaining nine were used, but eight showed spontaneous tuberculosis on autopsy.

In this connection it is of interest to note how in instances where the results are irregular investigators carefully give all the details they used to avoid spontaneous tuberculosis from natural sources, but either forget or ignore the like danger from adjacent animals inoculated with the bovine virus from man.

The Royal Commission tested the virulence of the bovine and human viruses by subcutaneous injection of both tissue emulsions, mostly with estimated doses, and cultures. They found that the bovine virus from cattle when the number of bacilli was adequate caused an acute progressive generalized tuberculosis ending fatally. Compared with these results they

find that the viruses of Group I. (bovine type) from man were equally virulent. The viruses of Group II. (human type), however, even in larger doses failed in all instances to cause a serious generalized tuberculosis. The results varied, the greatest effect being a local lesion at the site of injection with extension to the neighboring lymph node and a few scattered retrogressive tubercles in various organs. They have compared the results of injections of emulsions of tuberculous tissues with those of cultures and find that a much smaller number of bacilli are effective in the former.

The dissemination mentioned was present in about half the calves inoculated with human viruses, the lesions, however, were very small. Quoting Cobbett, "In a few calves these little lesions were so numerous that had the symbols been made to express the number and anatomical range of the tuberculous lesions independent of their size and severity, these cases must have come under the heading "general tuberculosis." On intravenous inoculation death was easily caused in contrast to the subcutaneous inoculation. The cause of death was the extensive involvement of the lungs, and occurred early.

The results of these two series of investigations are fairly comparable. In both types the Royal Commission caused a greater amount of tuberculosis in calves, but the differences shown between the two types are about the same. In a few instances they found some degree of intermediate virulence; these will be noted in the resumé of their cases (page 145).

It is an almost hopeless task to correlate all the experimental work that has been done, but these two very large series of experiments clearly establish the differences in the virulence of the two types. Considering this difference and the fact that in no instance has a culture been isolated directly from cattle, showing the characteristics of the human type, the question of possible infection by cattle from man under natural conditions is practically settled. Allowing every possible contention, one may admit that under certain circumstances of age of the animal, dosage,

method of inoculation and purity of the material, tuberculous material or cultures from man of the human type may cause serious lesions or death in the bovine animal. On the other hand, with pure material, or cultures and correlated dosage, the human virus is evidently different from the bovine virus in its capacity to cause disease. This difference is so marked that in practically every case it gives conclusive evidence of the type of bacillus used. Even those who combat the existence of two types are willing to admit that a culture which is virulent for calves is presumably of bovine origin.

Own observations. — It was out of the question for us to test every virus in calves. The work done by other investigators seemed to us so conclusive that only a limited number of viruses were tried. Furthermore, our results with cultures and rabbit inoculation were so uniform, the differences being so marked in the two sets of viruses from man, one of these being in every respect identical with viruses from cattle, that there seemed no necessity for further proof of the reliability of our classification.

The conclusions from our inoculation work with calves are parallel with those in rabbits. All viruses of the bovine type are capable of causing a tuberculosis of generalized nature, usually progressive and causing the death of the animal. The human type causes only a localized tuberculosis or extension to the neighboring lymph nodes, at most an occasional distant lesion may be found.

The methods employed were as follows: All calves were tested with tuberculin before use. The culture to be inoculated was prepared the same as for rabbit inoculations except that a suspension was made in which each cubic centimeter represented ten milligrams. Five cubic centimeters or fifty milligrams were inoculated. Subcutaneous inoculation in the side of the neck was used. In every case where a bovine virus was inoculated, rabbits were inoculated from the same emulsion as controls. As in the case of the rabbit autopsy, macroscopic lesions were relied upon. In a few instances

where exceptional lesions have apparently arisen we have used guinea-pig inoculations for verification. This evidence is not altogether beyond question. Negative results may mean healing, positive results may mean dissemination and persistence, without any relation to the doubtful lesion found.

Eighteen strains of the human type were inoculated, and only two of these gave any evidence of dissemination. In one calf there were some doubtful lesions in the lung which gave positive results in the guinea-pig. In another some abdominal lymph nodes were typical macroscopically, but failed to cause any lesions in the guinea-pigs inoculated with it. It is difficult to interpret these lesions, but they cannot at any rate be considered at most more than slight dissemination and were not extensive enough to be classed as a generalized tuberculosis.

Fourteen strains of the bovine type were also inoculated. Of these eight were from human sources. Six produced a progressive generalized tuberculosis of varying extent causing the death of the animal after a period of twenty-three to seventy-three days. One caused a widespread tuberculosis, but the disease was regressive and had ultimately no influence on the health of the animal. Another case showed practically no virulence when inoculated. The control rabbits showed that this strain had lost the virulence which it originally possessed for rabbits. The inoculations with the six bovine viruses from cattle gave closely the same results. Four caused progressive generalization with death in from twenty to ninety days, one caused a moderate regressive tuberculosis, but influenced the health of the calf. Another strain was known to have lost virulence in rabbits, but was inoculated into calves to verify the parallelism of virulence in the two animals.

The results have been practically what would be expected in the light of the results of other workers. The bovine viruses have shown a marked tendency to generalization, whereas the human viruses have shown practically none. The parallelism with the rabbit results is very close. One peculiarity is that although a virus may cause only a

regressive tuberculosis in calves, the inoculation into rabbits shows no evidence of regression.

The fact of parallelism is to be interpreted with certain precautions. This does not extend to the distribution of the lesions, that is, the organs involved.

Where calves are used for differentiation it is very essential that early cultures be used for inoculation. This is not always possible, as many bovine viruses give only a small amount of growth. This difficulty is an objection to the use of calves. A virus which is typical culturally, and in early inoculations, may fail to give results by the time it is inoculated into calves, but this in no way weakens the conclusion that the virus is of the bovine type. Because the majority of tubercle cultures have been found to be stable in their virulence, too much reliance has been put upon this fact with erroneous results.

With all the evidence at hand we consider the necessity of calf inoculations, except in very unusual instances, for differentiation of type as past. If the cultural and virulence tests in rabbits have not been successful the crucial time for differentiation in calves may have elapsed, not to mention the fact that the failure of the simpler methods may mean a lack of technic which would probably give equally poor results in calves.

The following gives a tabular summary of the results of inoculation, and a detailed description of each calf. Some of the human viruses were reisolated from calves by means of the guinea-pig to see if any change had taken place. These results are given and will be spoken of in the discussion of passage experiments.

TABULATION OF AUTOPSY FINDINGS.

Calves inoculated with human viruses.

Length of Life.	Intensity of Lesions Found and Number of Animals.					Total Animals.		Number Inoculated.
	Organ.	+	++	+++	++++	+	−	
Killed 140 to 167 days.	Lungs.	1	—	—	—	1	17	18
	Thoracic lymph nodes.	—	—	—	—	0	18	
	Spleen.	—	—	—	—	0	18	
	Liver.	—	—	—	—	0	18	
	Kidneys.	—	—	—	—	0	18	
	Abdominal lymph nodes.	(1?)	—	—	—	(1?)	17	

TABULATION OF AUTOPSY FINDINGS. — *Continued.*

*Calves inoculated with bovine virus from man.**

Length of Life.	Intensity of Lesions Found and Number of Animals.					Total Animals.		Number Inoculated.
	Organ.	+	++	+++	++++	+	—	
Died 23–73 days.	Lungs.	—	—	1	5	6	0	6
	Thoracic lymph nodes.	1	4	1	—	6	0	
	Spleen.	—	2	1	—	3	3	
	Liver.	3	—	—	—	3	3	
	Kidneys.	—	1	—	—	1	5	
	Abdominal lymph nodes.	2	2	1	—	5	1	
Killed 125 days.	Lungs.	—	—	1	—	1	0	1
	Thoracic lymph nodes.	—	—	1	—	1	0	
	Spleen.	—	—	1	—	1	0	
	Liver.	—	—	—	—	0	1	
	Kidneys.	—	—	—	—	0	1	
	Abdominal lymph nodes.	—	1	—	—	1	0	

*Calves inoculated with bovine viruses from cattle.**

Length of Life.	Intensity of Lesions Found and Number of Animals.					Total Animals.		Number Inoculated.
	Organ.	+	++	+++	++++	+	−	
Died 20–90 days.	Lungs.	—	—	—	4	4	0	4
	Thoracic lymph nodes.	—	2	2	—	4	0	
	Spleen.	—	1	—	—	1	3	
	Liver.	1	1	—	—	2	2	
	Kidneys.	1	—	1	—	2	2	
	Abdominal lymph nodes.	3	—	—	—	3	1	
Killed 134 days.	Lungs.	—	—	1	—	1	0	1
	Thoracic lymph nodes.	1	—	—	—	1	0	
	Spleen.	—	—	—	—	0	1	
	Liver.	1	—	—	—	1	0	
	Kidneys.	—	—	—	—	0	1	
	Abdominal lymph nodes.	1	—	—	—	1	0	

* Exclusive of two viruses, Milk No. 89 and Tub. Aden. Chil. No. 96, which had lost their virulence, as shown by control rabbits.

RESULTS OF CALF INOCULATIONS.

Diagnosis of Case.	No.	Autopsy.
Pulmonary tuberculosis. Adults.....	64. H. type. (See 125.)	Local tuberculosis. Calf 190 pounds. Killed 142 days. Gain 77½ pounds. Site of injection, completely caseous mass measuring 3 x 2 x 2 inches. Prescapular node on same side measured 2 x 1 x ¾ inches. No macroscopic lesions. No tuberculosis elsewhere. Site of injection, guinea-pig positive. Prescapular node, guinea-pig negative.
" " "	118. H. type.	Local tuberculosis and of neighboring lymph node. Calf 185½ pounds. Killed 140 days. Gain 83½ pounds. Site of injection, caseous mass measuring 3 x 1 x 1½ inches. Prescapular node on same side measures 3 x 1 x ½ inches and contains a small caseous area one-fourth inch in diameter, which is partly calcified. No other tuberculous lesions. Site of injection, guinea-pig positive. Prescapular node, guinea-pig positive.
Pulmonary tuberculosis. Children.....	15. H. type.	Local tuberculosis. Calf 119 pounds. Killed 142 days. Gain 55 pounds. Site of injection, caseous mass 2 x 1 x ¾ inches. Prescapular node swollen, no macroscopic tubercles. No other lesions. Site of injection, guinea-pig negative. Prescapular node, guinea-pig negative.
Tuberculous adenitis. Children.....	96. B. type.	Local tuberculosis. Calf 256 pounds. Killed 104 days. Loss 9 pounds. Site of injection, nothing remaining. Prescapular node, small fibrous tubercle with caseous point in its center. Culture injected, 19th generation. Rabbits inoculated with same emulsion, no lesions, with .01 milligram dose. This virus therefore had lost its virulence.
" " "	116. H. type.	Local tuberculosis. Calf 189½ pounds. Killed 142 days. Gain 83½ pounds. Site of injection, caseous mass, 2 x 2 inches. Prescapular node swollen, no tubercles. No other lesions.
" " "	150. H. type.	Local tuberculosis. Calf 218 pounds. Killed 142 days. Gain 66 pounds. Site of injection, caseous mass, 2 x 2 inches. Prescapular nodes swollen, no tubercles. No other lesions.

"	"	"	163. B. type.	Progressive generalized tuberculosis. Calf 156 pounds. Died 73 days. Loss 30 pounds. Site of injection, fluctuating mass, 7 x 5 x 3 inches, filled with yellowish fluid containing cheesy lumps. One portion, firmer than the rest, is a mass of caseous tubercles. Prescapular node, same side, enlarged, and shows caseous areas. Submaxillary nodes on each side contain few tubercles. Inguinal nodes, left, slightly enlarged and show one small tubercle each, right, ditto. Lungs, both studded with caseous tubercles about one-fourth inch in diameter, uniformly distributed, giving a shotty feel to lungs. Posterior mediastinal nodes enlarged, some being 2 x 1 x 1 inches in size and show many caseous areas. Mesenteric nodes enlarged and contain caseous tubercles, some of the nodes are 2 x 1½ x ¾ inches and completely caseous. Kidneys, surface covered with small hemorrhagic areas. No tubercles. Spleen, moderate number of yellowish caseous tubercles about one-eighth inch in diameter uniformly distributed. Liver negative. Portal nodes about 1½ x ¾ x ½ inches in size and studded with large tubercles. Lymph nodes about esophagus near stomach are enlarged and contain a few tubercles.
"	"	"	217. B. type.	Progressive generalized tuberculosis. Calf 203½ pounds. Died 23 days. Loss 69½ pounds. Site of injection, abscess, 8 x 4 x 4 inches, yellowish caseous material. Prescapular node, 4 x 1 x 1½ inches with several large caseous tubercles. Continuous with this is a chain of lymph nodes extending to the anterior and posterior mediastinum, which are involved. Lymph nodes, of both anterior and posterior mediastinum, measure from one inch to three inches and show a varying number of tubercles, some small, others larger with beginning caseation. Retroperitoneal and mesenteric nodes, many show small tubercles. Liver, moderate number of small tubercles. Spleen, many small areas, apparently tubercles, cannot be differentiated surely from connective tissue reticulum. Lungs, almost solid and riddled with small tubercles, some beginning caseation. Kidneys negative. Inguinal nodes enlarged, no tubercles.

RESULTS OF CALF INOCULATIONS. — *Continued.*

Diagnosis of Case.	No.	Autopsy.
Tuberculous adenitis. Children, *cont.* ...	262. B. type.	Progressive generalized tuberculosis. Calf 158½ pounds. Died 53 days. Loss 22½ pounds. Site of injection, cyst-like, contents completely broken down (5 x 4 x 1 inches), thin serous fluid, walls are caseous. Prescapular node, same side, large number of small tubercles showing caseation. Axillary nodes, same side, about one inch in diameter riddled with small tubercles. Bronchial and posterior mediastinal nodes enlarged, largest 3 x 2 x 1 inches, riddled with medium-sized tubercles, with beginning caseation. Lungs do not collapse, riddled with confluent tubercles, beginning caseation. Spleen enlarged, few tubercles in substance. Mesenteric nodes enlarged, many show small tubercles. Portal node enlarged and riddled with tubercles. Liver negative.
" " "	393. B. type.	Progressive generalized tuberculosis. Calf 184 pounds. Died 55 days. Loss 53 pounds. Site of injection, swelling 8 x 5 x 5 inches, infiltrating, yellow caseous center, broken down, with serous fluid. Whole chain of cervical nodes on same side enlarged, the largest being 3 x 2 x 1 inches; varying number of large caseous tubercles in each node. Prescapular node, 3 x 2 x 1 inches, a mass of confluent caseous tubercles. Axillary and inguinal nodes enlarged and contain numerous tubercles. Lungs riddled with shot-sized tubercles, showing beginning caseation. Smaller tubercles scattered over the pleura. Anterior and posterior mediastinal node, both chains completely involved, the largest being the bronchial, 3½ x 3 x 2 inches. All show tubercles, some being completely caseous. Spleen riddled with small tubercles showing beginning caseation. Pelvis of each kidney shows cheesy tubercles. Liver negative. Peritoneum, few tubercles. Mesenteric nodes enlarged, numerous tubercles. Retroperitoneal node enlarged, numerous tubercles. Portal node completely caseous.

Abdominal tuberculosis.	Children.....	109. B. type.	Progressive generalized tuberculosis. Calf 152 pounds. Died 24 days. Loss 31½ pounds. Site of injection, 10 x 6 x 2 inches, a cavity, the walls formed by semi-caseous material, the center filled with sero-purulent fluid. Prescapular node on same side, 3 x 2 x 2 inches, with many tubercles with beginning caseation. Mediastinal lymph nodes, anterior and posterior, few enlarged with small tubercles. Lung semicollapsed, very many small tubercles with beginning caseation. Spleen, possibly very fine tubercles. Retroperitoneal nodes, few contain small tubercles. Liver, moderate number of small tubercles.
"	"	"	155. H. type.	Local tuberculosis. Calf 263 pounds. Killed 142 days. Gain 109 pounds. Site of injection, caseous mass, 3 x 3 x 2 inches. Prescapular node enlarged, no tubercles. No tuberculosis elsewhere.
Generalized tuberculosis.	Children	19. H. type. (See 16.)	Local tuberculosis. Calf 262½ pounds. Killed 140 days. Gain, 95½ pounds. Site of injection, tumor 1 x 1½ inches, completely caseous. Prescapular and one posterior mediastinal node enlarged, no tubercles. Site of injection, guinea-pigs positive. Mediastinal node, guinea-pigs negative.
"	"	"	20. H. type. (See 19.)	Local tuberculosis and of adjacent lymph nodes. Calf 133 pounds. Killed 140 days. Gain 62 pounds. Site of injection, pocket surrounded by fibrous tissue, contains no caseous material; immediately adjacent are a few cervical lymph nodes, almost completely caseous. Prescapular node enlarged, no tubercles. Lungs well collapsed, small area of consolidation at ventral margin. Site of injection, guinea-pig positive. Prescapular node, guinea-pig positive. Lung, guinea-pig negative.
"	"	"	58b. H. type.	Local tuberculosis. Calf 129½ pounds. Killed 140 days. Gain 74½ pounds. Site of inoculation, 2½ x 1 inches completely caseous. Prepectoral node swollen, no tubercles. Lungs, few congested areas, atelectasis. Site of injection, guinea-pig positive. Prescapular node, guinea-pig negative.

RESULTS OF CALF INOCULATIONS. — *Continued.*

Diagnosis of Case.	No.	Autopsy.
Generalized tuberculosis. Children, *cont.*,	62. B. type.	Regressive generalized tuberculosis. Calf 125 pounds. Killed 125 days. Gain 81 pounds. Site of injection, 7 x 6 x 4 inches, completely caseous and surrounded by a number of small lymph nodes, about one-half inch in diameter and completely caseous. Prescapular node, 3 x 2½ x 1 inches, numerous tubercles of varying size; most are completely caseous. Inguinal node, left, enlarged numerous caseous tubercles one-fourth inch in diameter, two right, same. Anterior mediastinal node at entrance to thorax, 3 x 2 x 2½ inches, completely caseous. All the anterior and mediastinal nodes are enlarged and contain caseous tubercles or are completely caseous. Lungs, scattered, fairly numerous caseous tubercles about one-fourth inch in diameter. Liver negative. Several lymph nodes, one under surface, are caseous. Spleen, numerous one-fourth inch caseous tubercles. Mesenteric and retroperitoneal nodes enlarged and show few caseous tubercles. Kidneys negative. Some of the lymph nodes show beginning calcification.
" " "	74. H. type.	Local tuberculosis. Calf 157 pounds. Killed 140 days. Gain 47 pounds. Site of injection, tumor 2 x 1½ inches completely caseous. Prescapular node enlarged, no tuberculosis. Lungs, few collapsed and congested areas, atelectasis. Mass of lymph nodes in ligament at junction of two gastric pouches. These nodes were caseous. Site of injection, guinea-pig positive. Prescapular node, guinea-pig positive. Lymph nodes, guinea-pig negative. Whether these latter lesions were due to the injected material or regressive old lesions we cannot say. If they were the former we should expect to find tubercle bacilli by injection into pigs, which was not the case.
" " "	98. H. type. (See 97.)	Local tuberculosis. Calf 204 pounds. Killed 142 days. Gain 84 pounds. Local lesion, two masses each about 3 x 1½ x 1½ inches. Completely caseous. Prescapular node swollen, no tubercles. Site of injection, guinea-pigs positive. Prescapular node, guinea-pigs negative.

" " "	99. H. type. (See 98.)	Local tuberculosis. Calf 242½ pounds. Killed 142 days. Gain 70⅔ pounds. Site of injection, mass 2¼ x 2 x 2 inches, completely caseous. Prescapular node enlarged, no tubercles.
" " "	100. H. type. (See 98.)	Local tuberculosis. Calf 226 pounds. Killed 142 days. Gain 116 pounds. Site of injection, caseous mass, 2 x 2 x 2 inches in diameter. Prescapular node enlarged, no tubercles.
" " "	148. H. type.	Local tuberculosis. Calf 190 pounds. Killed 142 days. Gain 56 pounds. Site of injection, caseous mass, 1½ inches in diameter. Prescapular node enlarged, no tubercles.
" " "	377. H. type.	Local tuberculosis. Calf 158 pounds. Killed 167 days. Loss 5 pounds. Site of injection, nothing remaining except a small one-fourth inch nodule which exudes cheesy material on section. No tuberculosis elsewhere.
" " "	634. B. type. (See 651.)	Progressive generalized tuberculosis. Calf 247½ pounds. Died 31 days. Loss 69½ pounds. Site of injection, 4 x 3 x 3 inches, caseous and completely softened. Prescapular node, 6 x 4 x 4 inches, numerous small tubercles. Lungs completely riddled with small tubercles. Bronchial node enlarged, few small tubercles. Liver, moderate number of small grayish tubercles. Portal node enlarged, few small tubercles. Spleen enlarged, very small tubercles. Lymph nodes throughout perhaps swollen, but show no definite tubercles.
Tubercular meningitis	2. H. type. (See 4.)	Local tuberculosis. Calf 197½ pounds. Killed 142 days. Gain 69½ pounds. Local lesion, small area, about 1 x 1½ inches, caseous. Prescapular node enlarged, no tubercles, the same for the axillary on the same side. Lungs completely collapsed, bands of more solid tissue feel like strands of connective tissue. Atelectasis? No tubercles. Mesenteric nodes, several swollen, suspicious small areas. Liver, small area of degeneration. Site of injection, guinea-pig positive. Prescapular node, guinea-pig negative. Axillary node, guinea-pig negative. Lung, guinea-pig negative. Mesenteric node, guinea-pig negative.

RESULTS OF CALF INOCULATIONS. — *Continued.*

Diagnosis of Case.	No.	Autopsy.
Tubercular meningitis, *cont.*	61. H. type.	Local tuberculosis. Calf 109 pounds. Killed 140 days. Gain 133 pounds. Site of injection, small mass 2 inches in diameter, caseous. Prescapular node not enlarged. Lungs, several small areas of consolidation, no resemblance to tuberculosis macroscopically. No other lesions. Site of injection, guinea-pigs positive. Lung, guinea-pigs positive.
Tuberculosis of bones and joints	558. H. type.	Local tuberculosis. Calf 149 pounds. Killed 167 days. Gain 9 pounds. Site of injection 2 x 2 x 2 inches. Fibrous, small amount of caseous material, some calcification. No tuberculosis elsewhere.
Viruses from milk	89	Local tuberculosis. Calf 145 pounds. Killed 130 days. Loss 2 pounds. Site of injection, 6 x 4 x 2½ inches, fibrous, wall contents serous with caseous floccules. Prescapular node enlarged. No tubercles. Lungs collapsed, two small doubtful tubercles. This injection was done to confirm the loss of virulence in rabbits, which has already been described. For this reason it has no diagnostic value as to type.
Bovine viruses. Cattle	310	Progressive generalized tuberculosis. Calf 218 pounds. Died 20 days. Loss 55 pounds. Site of injection, mass 6 x 8 x 2 inches, yellowish on section, beginning softening. Prescapular node, confluent tubercles with beginning caseation. Lungs riddled with small grayish miliary tubercles. Lymph nodes, anterior and posterior mediastinal, and especially the bronchial, show small tubercles. Liver, a few scattered small tubercles. Spleen enlarged, suspicious tubercles throughout. Mesenteric nodes, all are swollen, few show small tubercles.

" " "	314	Progressive generalized tuberculosis. Calf 211½ pounds. Died 90 days. Loss 83½ pounds. Site of injection, 7 x 5½ x 4 inches, a caseous mass with caseous tubercles in surrounding tissue, the neighboring cervical nodes are likewise involved. Prescapular node, 5½ x 5 x 4 inches completely riddled with large yellowish caseous tubercles. Superficial nodes, in inguinal and intercostal region, contain few caseous tubercles. Lungs riddled with numerous caseous tubercles one-fourth to one-half inch in diameter. Pleura, parietal and visceral, shows numerous grape-like growths one-half inch to three-fourth inch in diameter, some pedunculated. Lymph nodes, anterior, and posterior mediastinal enlarged, contain caseous tubercles. Bronchial, 3½ inches long, completely riddled with caseous tubercles. Mesenteric and retroperitoneal nodes all enlarged, tubercles of varying size. Portal node about 2 inches in diameter and almost completely caseous from confluence of tubercles. Liver, numerous caseous tubercles, some one-half inch in size. Spleen, same. Kidneys, few tubercles.
" " "	327	Regressive generalized tuberculosis. Calf 188½ pounds. Killed 134 days. Loss 35½ pounds. Local lesion, 3 x 4 x 3 inches; fibrous wall with caseous material in center, beginning calcification. Prescapular node enlarged, no distinct tubercles, few doubtful areas. Prepectoral node on opposite side shows one distinct tubercle. Lungs, throughout both are fairly numerous small grayish tubercles, many are in small congested atelectatic areas. Many of the tubercles are gritty when cut. Lymph nodes, a few in posterior mediastinum show small tubercles, some with calcification. Liver, six small tubercles under the capsule, few doubtful tubercles in substance. Spleen negative. Kidneys negative. Mesenteric nodes, two or three show small tubercles.

Note.—The control rabbits injected with the same emulsion showed no recognizable loss of virulence over early inoculations.

RESULTS OF CALF INOCULATIONS. — *Concluded.*

Diagnosis of Case.	No.	Autopsy.
Bovine viruses. Cattle, *cont.*.....	334	Progressive generalized tuberculosis. Calf 193 pounds. Died 27 days. Loss 150½ pounds. Local lesion, mass 5 x 2 x 3 inches with caseation center completely softened. Prescapular node contains numerous tubercles. Lymph nodes, posterior mediastinal and especially the bronchial, show numerous tubercles. Majority of nodes are small but vary up to 5 x 1 x 1½ inches. Those of abdomen negative, except portal node, which shows a few small tubercles. Lungs only partially collapsed, riddled with small tubercles. Abdominal organs negative.
" " "	491	Progressive generalized tuberculosis. Calf 121 pounds. Died 40 days. Loss 11 pounds. Site of injection, caseous mass 6 x 3 x 2 inches. Close to it are two cervical nodes, which are enlarged and show large tubercles with beginning caseation. Prescapular node enlarged, confluent tubercles replacing glandular tissue completely, beginning caseation. Inguinal node, left side, few small tubercles. Mediastinal nodes, anterior and posterior, all enlarged, the largest being 2½ x 1½ x ½ inches, showing numerous medium-sized tubercles with beginning caseation. Lungs riddled with large caseous tubercles, there being practically no crepitation. Liver and spleen, no macroscopic tubercles. Kidneys, both show many medium-sized tubercles in capsule and cortex. Both are distended, condition of hydronephrosis. Retroperitoneal nodes enlarged and some show small tubercles. *Note.* — In some of the preceding calves inoculated with bovine viruses one could not tell whether tubercles were present or not in the spleen. When these were sectioned, tubercles were found which were large enough to be seen in the stained section with the naked eye or were microscopic. These are not included, as only distinct macroscopic lesions were considered in comparisons.

Cultures after passage through one calf. — The following strains were isolated from calves injected with these viruses. This was done to see if any change had taken place in cultural characteristics or rabbit virulence during the residence in the bovine tissues. As is evident from the table no change had taken place. The reisolation was in each case through the guinea-pig.

Diagnosis of Case.	No.	Isolated from.	Cultures.	Rabbit Virulence.	Notes.
Pulmonary tuberculosis. Adults	64	Site of injection.	H. type 1 (?). (Potato not planted.)	Not tested.	No change.
" " "	118	" " "	H. type 1.	" "	" "
	118	Prescapular node.	" " 1.	" "	" "
Generalized tuberculosis. Children.....	19	Site of injection.	" " 1.	" "	" "
	20	" " "	" " 1.	" "	" "
	20	Prescapular node.	" " 1.	Non-virulent.	" "
" " "	58b.	Site of injection.	" " 1.	Not tested.	" "
" " "	74	" " "	" " 1.	" "	" "
	74	Prescapular node.	" " 1.	Non-virulent.	" "
Tubercular meningitis. Children	98	Site of injection.	" " 1(?). (Potato not planted.)	Not tested.	" "
" " "	2	" " "	H. type 1(?). (Potato not planted.)	" "	" "
" " "	61	" " "	H. type 1.	" "	" "
	61	Lung.	" " 1.	Non-virulent.	" "

Correlation of results. — There is very little to be said concerning the results of inoculation of calves where known doses have been used. Comparing two large series of experiments, such as the German and English, there are some differences noticeable. The latter obtained more marked results upon the inoculation of human viruses, but their results were also more marked with the bovine viruses. What influence races of cattle may have in causing such variations we do not know, though there is evidence of difference in susceptibility to infection shown by different races. The essential point, however, is not what lesions this or that investigator has caused by the inoculation of a virus, but rather are the lesions in accord as to the differences of virulence in cattle and the value of this difference for differentiation. In this respect there is a very close agreement. Our own results are so uniform that considering them in connection with the total work that has been done, the evidence is practically conclusive both as to the difference in virulence and its value for differentiation of type. The few instances where results have apparently been in contradiction to the total findings stand as probable cases with insufficient controls. These aberrant tests can never be repeated, but as the favorable evidence grows they become less and less credible and tend to fall into the class of experimental errors.

DIFFERENCES IN VIRULENCE IN OTHER ANIMALS.

Differences have been noted in various animals. The general rule is that the bovine virus is more virulent than the human virus. These differences are more of academic interest than of practical value for differentiation of type. In some instances these differences in virulence run as in the calf parallel according to the type naturally infecting these animals.

None have been as extensively used for differentiation as the calf and rabbit. The latter being a small animal, the quarters that are necessary are small. Recently Trommsdorff has suggested the intravenous inoculations in the mouse for differentiation. There can be no advantage in this except

where the size of the animal is a factor. We have not done any experimental work along these lines, nor can we see any advantage for greater certainty in the use of more than one species except, possibly, in a very few unusual cases, to differentiate a virus. In fact as is evident from our descriptions even the calf is necessary only in very exceptional cases, if at all. This work has been necessary and important in laying the basic work on the differentiation of type in that it shows that where differences in virulence existed, these differences were uniform according to type and not differences in the members of one type alone.

VIRUSES SHOWING VARIANT CHARACTERS (ATYPICAL VIRUSES).

The finding of variant strains by different investigators has received undue attention. This was due to their use as evidence against the essential division into two types or groups, and the stability of these types or groups. If these variants were atypical or transitional to such an extent that they could not be differentiated, grouping would fail and the application to practical tracing of types of infection would lose most of its value. Most of the so-called atypical strains can however be classified from the protocols given by the different investigators.

We have spoken of the variant characters under cultural characteristics and rabbit virulence. The following is a tabular summary of the results in one or the other characteristic of every virus or strain which showed minor or marked variations:

TABLE.

Diagnosis of Case.	Number and Type.	Cultures.	Rabbit Virulence.
	13. H. type 1.	Typical.	1 mg. Few tubercles in spleen.
	22. " " 1.	"	1 mg. Tubercular (?) inguinal node.
	27. " " 1.	"	1 mg. Few tubercles in spleen.
	36. " " 1.	"	1 mg. Many tubercles in spleen.
	76. " " 2b.	Poor on potato.	Typical.
	105. " " 1.	Typical.	1 mg. Tubercular peritonitis.
	118. " " 1.	"	.01 mg. Axillary and inguinal nodes show tubercles.
	232. " " 1.	"	1 mg. Tuberculous (?) tarsal node.
	286. " " 1.	Poor in original tubes.	Typical.
	307. " " 1.	Poor in original tubes.	"
	341. " " 1.	Poor in original tubes.	"
	366. " " 1.	Typical.	1 mg. Tuberculous inguinal node.
	371. " " 1.	Poor in original tubes.	Typical.
Pulmonary tuberculosis. Adults.	407. " " 2a.	Poor on potato.	"
	414. " " 1.	Poor in original tubes.	"
	415. " " 1.	Typical.	1 mg. Tubercles (?) in axillary and inguinal nodes.
	419. " " 1.	"	1 mg. Tubercles in axillary nodes.
	421. " " 2b.	Poor on potato.	Typical.
	425. " " 1.	Poor in original cultures.	"
	437. " " 2a.	Slow on potato.	"
	459. " " 1.	Typical.	1 mg. Tubercles in intestine.
	485. " " 1.	"	.01 mg. " " "
	500. " " 2a.	Poor on potato.	Typical.
	516. " " 2b.	" " "	"
	517. " " 2b.	" " "	"
	539. " " 2a.	" " "	"
	545. " " 2a.	" " "	"
	566. " " 2a.	" " "	"

TABLE. — *Continued.*

Diagnosis of Case.	Number and Type.	Cultures.	Rabbit Virulence.
Tuberculous adenitis. Adults.	619. H. type 1.	Poor in original cultures.	Typical.
	646. " " 1.	Typical.	.01 mg. Spleen, two tubercles.
Tuberculous adenitis. Children.	18. H. type 2a.	Poor on potato.	Typical.
	94. " " 1.	Poor in original cultures.	"
	184. B. type 1.	Typical.	1 mg. Acute death without generalization.
	217. " " 1.	"	1 mg. Acute death without generalization.
	272. H. type 1.	"	1 mg. Tuberculosis of bones. .01 mg. Tuberculosis of node?
	381. B. type 1.	"	1 mg. Acute death without generalization.
	396. H. type 1.	"	1 mg. Tuberculous axillary and inguinal nodes.
	670. " " 1.	Poor in original tubes.	Typical.
Abdominal tuberculosis. Children.	109. B. type 1.	Typical.	1 mg. Incomplete generalization.
	604. H. type 1. (See 155.)	"	1 mg. Spleen and inguinal nodes. Tubercles?
Generalized tuberculosis. Children.	1. H. type 2a.	Poor on potato.	Typical.
	45. " " 2a.	" " "	"
	90. " " 1. (See 97.)	Typical.	1 mg. Spleen, two tubercles.
	122. B. type 1.	"	1 mg. Acute death, no generalization.
	123. " " 1.	"	1 mg. Acute death, no generalization.
	274. H. type 1. (See 273.)	"	1 mg. Tubercles of axillary nodes.
	276. H. type 1.	Poor in original cultures.	Typical.
	377. " " 4.	Poor on all media.	"
	389. " " 2a. (See 388.)	Slow on potato.	"
	434. H. type 1.	Both poor in original cultures, slower increase than usual.	"
	435. " " 1.		
	689. " " 1.	Typical.	1 mg. Tubercles in intestines.
	692. " " 1.	"	1 mg. Spleen, numerous tubercles, axillary and inguinal nodes involved.

TABLE. — *Concluded.*

Diagnosis of Case.	Number and Type.	Cultures.	Rabbit Virulence.
Tuberculosis of bones and joints.	202. H. type 4.	Poor on all media.	Typical.
	558. " " 3.	Very moderate on all media.	"
	586. " " 1.	Typical.	1 mg. Tubercles of intestines.
	662. " " 1.	"	1 mg. Axillary and mesenteric nodes, tubercles. Spleen, one tubercle?
Genito-urinary tuberculosis.	621. H. type 1.	Typical.	1 mg. Tubercles of inguinal node.
	649b. " " 1.	"	1 mg. Spleen, two tubercles; liver, possible tubercles. Coccidiosis (?)
Miscellaneous.	661. H. type 3.	Very moderate on all media.	Typical.
Viruses from milk.	89. B. type 1.	Typical.	Lost virulence.
Bovine viruses. Cattle.	271. B. type 1.	Typical.	One rabbit lived too long; dose too small?
	311. " " 1.	"	1 mg. Death acute, no generalization.
	314. " " 3.	Very rapid increase, irregularities in strains.	1 mg. Generalization incomplete.
	327. " " 3.	Very rapid increase, irregularities in strains.	Typical.
	330. " " 1.	Typical.	Rapid loss of virulence.
	334. " " 2.	Better growth than usual in the early generations.	1 mg. Generalization incomplete.
	493. " " 1.	Typical.	.01 mg. Slower generalization than average.

We see no reason to consider seriously all these viruses as transitional forms. The great majority show such slight variation that they can be dismissed at once. Cases in which the one milligram dose has shown tendency to cause some generalization would be considered by some as indicative of transition. It is evident from what has been said under rabbit virulence that this is not the case. The few cases

where the .01 milligram showed variations are not unusual. It is far more striking that biological methods should give us such uniform results.

Narrowing down to those viruses which showed the closest relationship, we have the few cases where a bovine virus grew somewhat better than usual or increased very rapidly in amount of growth, or where a human virus grew more sparsely than the type or increased slowly from poor original cultures.

In these cases we have a distinct tendency to bridge the gap between the two types considering the cultural characteristics only. But even in these cases differentiation was possible and the predictions as to virulence were fulfilled.

If we compare our variations with those found by Rabinowitsch, Beitzke, Fibiger and Jensen, and others, it is evident that the majority of their atypical cases fall into one group or the other. It is impossible to discuss all the atypical or variant viruses reported, but in the comparison of our results with those of other authors under the different factors used in differentiation we have discussed their variant results.

It is hardly necessary to point out again that variation in one character is seldom accompanied by parallel variation in others, in other words, there is no parallelism beyond that found according to type. For this reason if the evidence is complete, even though one characteristic shows variant results, the sum total evidence leaves no doubt in our minds as to the type of organism.

Unfortunately not all of the so-called atypical viruses have been completely controlled. In some instances all the necessary precautions we now know to be necessary have not been observed as regards testing in the early generations. In others one cannot but wonder whether the worker was dealing with a pure culture. In either case it is certainly not justifiable to consider these cases as evidence against the reliability of the methods of differentiation. An example of this is one case reported by Lydia Rabinowitsch in a monkey. The cultures from this case (No. 11) grew like a bovine virus in the first generation, the following generations having the

characteristics of a human virus. The two cultures from the lung and spleen were tested on rabbits. All of these rabbits show an amount of dissemination more marked than one would expect from a human virus, but less than that caused by a bovine virus. A reisolation was done from one rabbit but only from the local abscess at the site of injection. No further note is made of this culture than that it grew slowly and sparsely. One wonders why its virulence was not tested. With the knowledge we now possess, one would never have allowed this condition in the rabbits to pass without reisolations from the internal organs and a study of the culture thus isolated. The possibility of a bovine fraction is for this reason not excluded. That the greater part of the culture was surely of the human type is evidenced by the fact that one rabbit with a dose of twenty milligrams survived and was killed after seventy-seven days. Furthermore, such irregularities would certainly warrant the inoculation of calves and reisolations from these animals to thoroughly clear up the question. We do not wish to state positively that this is a mixed culture, but only that this possibility is not excluded and that further work would have been desirable.

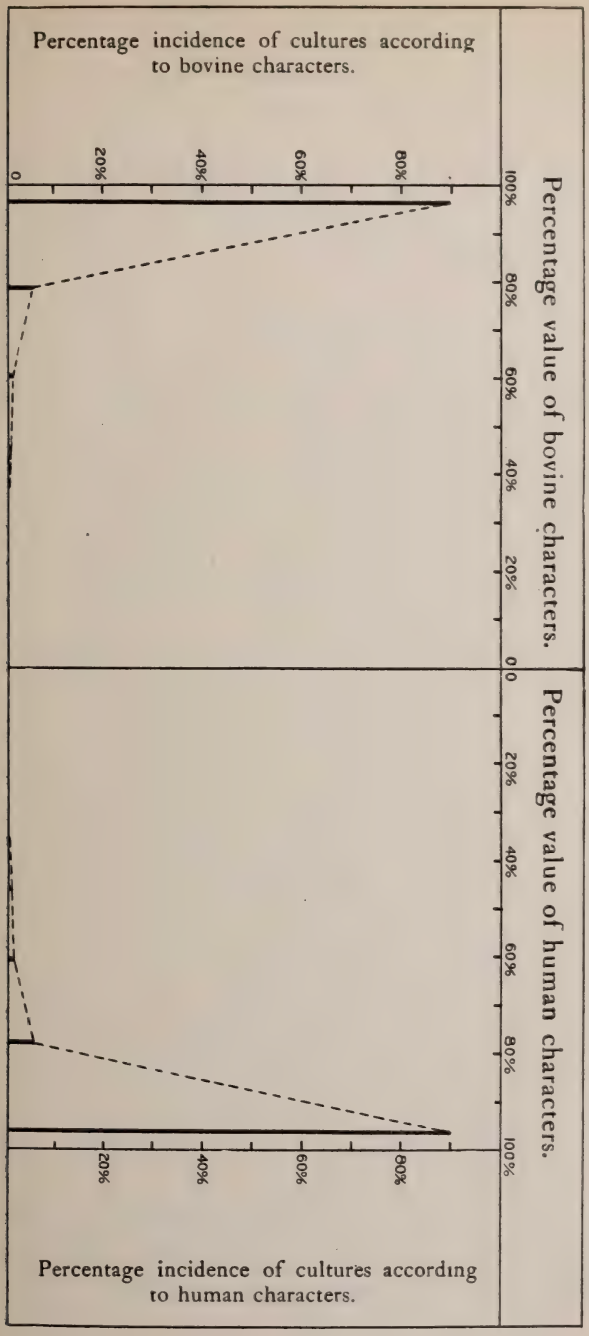
Recently a peculiar culture has been isolated. Shröder in examining sputa according to Spengler's method inoculated eleven sputa, showing the so-called *Perlsucht* forms, into rabbits. One of these caused marked lesions. The material was passed through a second and third rabbit with like results. Mietzsch isolated cultures from the third rabbit. These grew very freely and would be culturally classed as of the human type. Three rabbits inoculated from this culture again showed disseminated lesions. Inoculations into calves, however, caused only a local tuberculosis. Dieterlin also examined the culture and inoculated rabbits and a calf with the same results except that the virulence for rabbits seems somewhat lessened. This may have been due to the increased age of the culture. Möller examined three samples of sputum from the same case and in each instance the results were in no way irregular and conformed to those of a typical human virus. It is worthy of note that even with the dissemination

present in the rabbits, none showed fatal generalization. The culture is being further studied.

What interpretation one is to put upon such findings in the light of three subsequent examinations with different results one is unable to say. A mixed culture may be present, but one would expect that the human fraction would have been eliminated by the rabbit passage. Another possibility is that it is a human virus showing an excessive capacity to generalize. Whatever the condition, the contradictions shown by subsequent samples of sputum from the same patient should make us careful in drawing conclusions.

The two cases cited might be considered as evidence of unreliability shown by the rabbit. Assuming each to have been surely a human virus with no admixture, they then would only show the possible range of variation with a certain dose, and the possibility of close approach of the two types in one characteristic. As we have already stated, we cannot appreciate the arguments of certain workers that this is evidence of the non-existence of two types. The insistence on stereotyped differences in bacterial species and especially in varieties can no longer be maintained.

The work which has accumulated recently on the systematic relationship of bacteria and above all the study of the Coccacaeæ from the biological standpoint by Winslow shows the fallacy of the common conceptions of classification. Because of this we think the use of such terms as atypical or intermediate strains gives a false impression, viz., that these strains do not fall into one or the other group but form an indefinite intermediate group. As a matter of fact these strains, where sufficient information is obtainable, vary in one characteristic, very rarely in two and with all the evidence complete fall into one group or the other, though occupying a position somewhere between the two extreme types. If we were to graphically show our opinion of the condition present, giving each character a percentage value and considering the numerical incidence of cultures according to the total percentage value of all the characteristics, the condition would be roughly shown by the following curve.


Percentage incidence of cultures according to bovine characters.
0
20%
40%
60%
80%
100%
80%
60%
40%
20%
0
Percentage value of bovine characters.
0
20%
40%
60%
80%
100%
Percentage value of human characters.
Percentage incidence of cultures according to human characters.

Considering the close relationship of the two types the variations from the two extremes are not surprising. That the great majority of viruses so clearly group themselves around two extreme points and the absence of complete and gradual gradation of one type into the other is far more astonishing. This constitutes the clearest possible evidence of the existence of two types or groups and the reliability of the methods of differentiation.

CORRELATION OF CULTURAL CHARACTERISTICS AND VIRULENCE.

In general there is a parallelism between cultures and virulence when the two types alone are considered; roughly, virulence is inversely proportional to the amount of growth when a comparison is made of the results found in the early generations. This, however, only holds good when viruses are considered according to groups, and is subject to exceptions when individual variations are encountered. That we have been able in practically every case to predict the virulence of a virus from its cultural characters is sufficient proof of the fundamental reliability of this parallelism.

When we consider variations found in individual viruses this no longer holds. Thus a human virus of the poorer growing type is no more virulent than those showing maximum luxuriance, nor is a better growing bovine virus any less virulent than the most dysgonic virus of this class. Further increase in luxuriance of a bovine virus does not show parallel loss of virulence, nor loss of virulence equally marked increase in amount of growth. Any attempt to correlate such variations is, and has been, sure to lead to error.

All viruses from man and cattle therefore fall into two types; according to type cultural luxuriance is inversely proportional to animal virulence. Variations may occur in either characteristic, but without parallel variation in the other.

NECESSARY DATA FOR DIFFERENTIATION OF TYPE.

Exclusive of calf virulence which we have found unnecessary in our cases for differentiation, the culture and rabbit

gives us all the information needed for the differentiation of type. In the great majority of cases the question can be settled by cultures alone. The following method seems to us all that is necessary for differentiation. Assume that one is investigating a series of cases, purely for statistical purposes.

All cultures showing maximum luxuriance on glycerine egg in primary cultures are surely of the human type. This conclusion stands out in our mind as the most absolute cultural deduction. Successful vigorous growth on potato or marked pellicle formation in the next generation are confirmatory but unnecessary. This disposes of the majority of cultures in any series of cases unless selected.

The deductions as to type of the remaining viruses will be reliable in direct proportion to the experience of the investigator. In the absence of such experience immediate virulence tests had better be made.

All cultures not showing maximum luxuriance should then be transferred to glycerine egg, potato and glycerine egg with bouillon added. Cultures which have failed on glycerine egg should also be transferred on plain egg as glycerine egg may again fail. All cultures showing maximum luxuriance on this transfer are again surely of the human type and can be excluded from further observation. The cultures which are very sparse and have shown reluctance or negative results on glycerine egg are probably bovine.

All cultures showing any intermediate characteristics should be immediately tested on rabbits, using .01 milligram intravenously. Each virus should be injected into two rabbits at least. One rabbit may be killed between thirty-five and forty-five days if death does not occur. If generalized tuberculosis is present the virus is of the bovine type. If there is no generalization and a gain in weight, the other rabbit or rabbits may be allowed to live and killed between forty-five and sixty days; these will then almost surely show no lesions outside of the lungs or kidneys and the virus is surely of the human type. If a chance localization is present elsewhere this is ruled out as evidence of ability to generalize,

by the findings in the other rabbits. An occasional bovine virus may be slow in its generalization. This will probably not occur when tested so early. Further cultivation of the virus will in either case be confirmatory. This leaves us the very sparsely growing viruses, which are in all probability bovine. But there may be reluctant human viruses among them. Further cultivation would reveal these by a rapid increase in luxuriance or some would have increased already though not completely and would be tested among those showing intermediate characters on the second generation. The question of further cultivation brings in the possibility of loss of virulence. It seems, however, safe to make the virulence tests on the third or fourth generation at the latest. If on further cultivation any virus shows rapid increase in amount it should be tested in rabbits to rule out any possibility of mistaking a slower increasing human virus with a rapidly increasing bovine virus.

This leaves the viruses, which are persistently sparse for three or four generations; these are surely of the bovine type. The only possibility of error is that of failure of technic.

Our conclusions, then, are that all viruses showing maximum vigor in the first or second generation may be surely classed as of the human type. The deductions from cultural characteristics of the remaining viruses will be reliable in direct proportion to one's experience with fresh tubercle cultures. Failing this, or when time is a factor, rabbit inoculation should be resorted to with the latter viruses, using at least two rabbits for each virus.

The question of deduction from autopsy results has been fully discussed. With the above routine there are two possibilities of error; one is overlooking an avian culture. Avian infection in man is an open question. If true infection with this type of bacillus occurs in man the cases must be exceedingly uncommon. An interpretation of finding such as those of Rabinowitsch, who isolated this type from closed lesions, is to be made with caution, without further knowledge.

Whether there is any simple method by which one could

immediately recognize an avian culture, if one were encountered, we cannot say. We hope, however, to investigate a series of cultures according to the above routine to determine this point. The one fresh avian control we have used was distinctive.

The other possibility of error is a mixed infection, that is, a vigorously growing culture which contains a bovine fraction, which would only be shown if virulence tests were made. These cases must be exceedingly rare, only one being definitely proven, though there is a suspicion that such a condition existed in some of the unusual cultures reported. If, however, cultures from different organs are examined separately this would introduce a very small error.

As is evident, such a routine would not give all the information about an individual virus, but if a series of cases were examined in this way to establish further statistical data, we do not think that with efficient technic error could possibly be introduced amounting to more than a very small fraction of one per cent.

Although we do not wish to say that calf inoculations are never necessary to aid in the differentiation of type, still we must again point out that it was never necessary in our large series of cases.

PASSAGE EXPERIMENTS.

Critics of the conclusion that mammalian tubercle bacilli are separable into two types have attempted to prove their essential identity by passage experiments. Although broadly, all agree that differences are present, they assume that these differences are only unstable variations due to residence in different hosts and are susceptible to rapid change.

One point is lost sight of in these experiments. There is a great deal of difference between increasing the virulence of tubercle bacilli by passage and changing the typal characteristics. A moderate change of virulence in the body of a different species is a possibility which the most radical contender for typal differentiation would concede. As already shown, there is a great deal of variation shown by individual

viruses and, unfortunately for the almost naive conclusions of some investigators, in individual animals. Thus, when a virus is carried through a series of animals, and there is an apparent increase in virulence, the result in the latter animals is not controlled by determining the average virulence by using a large number. Even the original virulence is not tested in this way. Without correlation of dosage the experiments are not sufficiently controlled to lead to definite conclusions.

When, however, an apparent transformation of type occurs, we are dealing with something more tangible. If a human virus is inoculated into cattle and later from this animal a bovine virus is obtained there can be no question that a new infection or a transformation has taken place. The question is then one of methods. As will be evident from the following, the most surprising thing is the apparent sudden transformation in some cases while little or none occurs in others.

It is impossible to discuss all phases of the subject. Admitting the possibility of change of virulence, there is one fact that seems to us to prove that marked change does not take place naturally. This fact is the correlation of characteristics shown by cultures. If a change of virulence occurred naturally in cattle infected from man, assuming this to be possible, and in man from cattle one should be able to cultivate eugonic but virulent viruses from cattle and dysgonic and non-virulent viruses from man. This is not the case and even considering all the variants there is difference in each characteristic and parallelism when these characters are compared. The possibility of transformation of type is, therefore, mainly considered rather than the single characteristic of virulence alone and critical summaries of the most representative experiments are given.

Von Behring, Roemer and Ruppel found that ten months' residence in the body of a goat changed a human virus from sputum so that it became virulent for cattle and so dysgonic that cultivation from the spleen of the goat failed and was successful only after passage through three guinea-pigs. Growth was very sparse and morphologically there was a

change to short forms. Roemer gives further details and admits such a change is unusual and that they have not been able to duplicate the results with other cultures. Another passage with the same culture showed no change after recovery from a goat one and one-half years after inoculation. The goat was inoculated also with the Arloing homogeneous culture. Likewise reisolations from a sheep after four weeks and from an enucleated eye of a cow eight weeks after intraocular injection showed no change.

Dammann and Müssemeier claim to have completely changed the type of a sputum culture by passage through goats. They used five goats, and give the impression of a gradual change which is not proven by the facts. From the second goat *not tested with tuberculin* a culture of the human type was isolated from an esophageal node, but a caseous lesion from the lung was used, concerning the nature of which they had no information to inoculate the next goat. The goat *was tested with tuberculin July 28, 1903, and inoculated April 11, 1904.* This goat and also the fourth and fifth died of generalized tuberculosis. The goats were injected with the tuberculous tissue from the preceding goat and not with culture. Cultures from the third goat resembled more a bovine culture, those from the fourth and fifth goat, tested culturally more fully, were typically bovine in character, and the last, which was the only one tested for virulence, was fully virulent. Any suggestion that the last three animals showed a gradually increasing virulence as evidenced by the individual goats, is fully met by the fact that the dosage was not measured and only one animal used which would not rule out variations in individual animals. In our estimation this is another supposed instance, not of increase of virulence, but a complete and sudden transformation of type. All the precautions to be observed in buying young animals, sterilizing the food, separation from all possibility of infection from cattle material are absolutely insufficient when animals inoculated with a virus of bovine type from man are present. Furthermore, there is a strong

suspicion that a certain percentage of their animals were naturally infected with tuberculosis.

De Jong reported a like increase in virulence after residence in a goat for three years and one hundred and forty-five days.

Eber's results stand apart in many ways and for this reason we give a short, critical summary of his results (see page 151) of the examination of cases of tuberculosis, together with a description of his passage experiments. The great irregularity of his results are thus more clearly evident than if they were separated and discussed under different headings. Because of the methods and the almost uniform success, that is, apparent demonstration of increased virulence in every attempt compared with the inherent contradictions of certain results, we do not feel that the work is convincing.

The Royal Commission gives the facts concerning some passage experiments, some with positive, some with negative results. In one case (16 J. H.) the tuberculous synovial membrane from a knee of a thirty-eight-year-old patient was inoculated into calves. The tuberculous lesions resulting were then inoculated into a second calf. Both these calves after sixty-seven and eighty-one days respectively showed only a local lesion with extension to the regional nodes. The first calf also showed a calcareous bronchial node, but this was not used for inoculation. The next or third calf showed more extensive lesions, there being many tuberculous nodules in the lungs and a few in the spleen and liver. Emulsions of the prescapular node of this calf caused a moderate but fatal generalized tuberculosis in one calf, a moderate generalized tuberculosis in another calf and fatal generalized tuberculosis in a rabbit. The passage was continued further, all the animals developing a moderate to severe generalized tuberculosis, according to the dose.

Another passage, however, of the original material through two calves and of the original culture through three calves showed no increase in virulence.

Cultures isolated from the first and third calf and from guinea-pigs receiving the original material had the characteristics

of the human type. Cultures after passage were typically bovine. There are some discrepancies present, however, and the one which strikes us most is that the culture from the prescapular node of the third calf, which shows the first marked evidences of dissemination, is listed by Hutchens as giving fair growth on serum but very good growth on glycerine serum, and in subculture is a typical human virus. Subsequent isolations from later calves varied in their luxuriance, but the fluctuation was within the limits of the bovine type.

Although this is open to more than one interpretation we wish to point out that the very first calf shows a suspicious lesion, viz., in the bronchial node, of the nature of which we have no information. Although this was not used it is possible that this calf was spontaneously infected and that there was a bovine element carried on and gradually increased in amount in the further passage. Or, on the other hand, was the third calf spontaneously infected? The cultures isolated from this calf, after ten months' cultivation, caused some lesions in the lung of a calf, in another calf there was no tendency to spreading beyond the regional nodes and little virulence evidenced in rabbits. Yet the original material from which these cultures were isolated was virulent in rabbits and calves. Was there a bovine fraction which failed to develop in cultures sufficiently and which on further cultivation was lost? If we knew the character of the bacilli in the internal lesions of the early calves we could draw deductions; without them the case stands unproven.

Another case (13 A.D.) has no information as to the nature of the original material, viz., tuberculous bronchial nodes and spleen of a child aged four years. The first culture is from a second calf inoculated with the material from the last of a passage series through three sets of guinea-pigs from the first calf. Tissue emulsions from this calf were virulent, yet cultures from the same organ showed the characteristics of a moderately growing human type. Further, after fifteen months' cultivation, the culture showed some virulence, one calf had some involvement of the lungs and bronchial node,

and there was some capacity to generalize in rabbits. This case seems to give nothing convincing. In the first place the bronchial node and spleen were inoculated together, with no information as to the character of the virus in the individual nodes. From the peculiar contradictions we feel justified in assuming the possibility of mixed infection in the original material in the absence of proof to the contrary.

The result of the feeding of mixed sputa (17 Sp. B.) and the subsequent passage of the material from the four calves is likewise inconclusive. A culture from the second calf in one series was extremely eugonic. In a second series of two calves and two sets of goats, with intervening guinea-pigs, there was no change in virulence. Likewise another passage through three calves showed no change. Positive results were obtained, therefore, in one of four series of passages. In this series the third calf seems to have been the point of change. This calf eighty-eight days after intravenous inoculation showed cavities and nodules in the lungs, caseous nodules in the thoracic nodes, two tubercles in the liver and tuberculous iritis and tubercles in the choroid. The control rabbits when killed showed relatively slight lesions even in one inoculated intravenously. The very next calf killed when dying after eighteen days showed an acute miliary tuberculosis, the control rabbits also dying of acute miliary tuberculosis. The virulence throughout the rest of the series was that of a bovine virus Cultures isolated from the sixth and eighth calves in the series were typical of the bovine type. There is one marked discrepancy. The culture from the rabbit inoculated from the fourth calf was distinctly eugonic, but highly virulent. All these factors make us think that the third calf harbored bovine tubercle bacilli and that on further intravenous inoculation a mixture was carried on and that the human fraction persisted for a while, due to the method of inoculation, viz., intravenous, but further on this was weeded out and the bovine fraction only remained. Such an assumption is justified in absence of proof to the contrary.

The Commission performed other passage experiments without success, and conclude that a change is not an invariable

result of passage. As is evident from the descriptions given even with the same virus success was not uniform.

The objections made to the results may appear to some to be over-critical. But in view of the numerous pitfalls inherent in the method we feel justified in demanding detailed and complete controls of every step in the process and, above all, a culture from the individual lesions of each animal with careful determination of its characteristics culturally and at least its virulence for rabbits in very early generations. If these do not coincide the possibility of a mixed culture should be absolutely excluded before any conclusions can be drawn. When, as under the conditions present on the grounds used by the English Commission, calves injected with bacilli which are virulent for them, although coming from man, are kept on the same premises as calves receiving bacilli which are non-virulent, it is extremely difficult and perhaps impossible to prevent the dissemination of the virulent bacilli.

Attempts at approaching this question from the human standpoint have been made by Fibiger and Jensen, and Dammann and Rabinowitsch. In the case reported by the former authors, a slaughter-house infection was followed after about five years by pulmonary tuberculosis and tuberculosis of kidney, ureter, prostate, and testicles. The involvement of the axillary and supra-clavicular nodes gives an appearance of continuity to the disease process. Of cultures from the skin, urine, testicle, and lung only the first showed bovine characteristics. The assumption that bacilli in the skin remained bovine and that the others lost this type because of the different soil is only an assumption, for which there is not one iota of proof. It certainly seems more reasonable to consider this a grafting of human infection on a case already infected with the bovine bacillus.

The case reported by Dammann and Rabinowitsch is also a culture from a slaughter-house infection. Following a cut, the fourth metacarpal bone became tuberculous, the process extending to the cubital and axillary nodes. Cultures isolated from operative material not quite nine months after the injury showed the characteristics of the human type.

The sequence in this case is more apparent than real. Because most injuries of this type when followed by tuberculous lesions are due to bacilli of the bovine type does not exclude the possibility of infection of the wound from human sources or that this case might not have been a hematogenous infection subsequent to the injury which determined its localization.*

Rabinowitsch makes assumptions of the same character in regard to a culture of the human type isolated from milk. This she regards as having come from the cow because some other cultures from milk were "atypical." With our own experience with the cultures from a guinea-pig inoculated with milk by Dr. Hess, we see no justification for this assumption. Under these conditions there are too many sources of error to draw inferences in variance with the uniform results from material directly obtained. Zwick approached the question in the most logical manner, viz., if such conditions exist one should examine the autopsy material from cattle and be able to find such cultures in at least a small percentage of cases. This, however, has not occurred.

The workers of the *Gesundheitsamt* have had absolutely negative results in effecting transformation in their passage experiments. Two passages, one through five goats totaling two hundred and eighty-four days, another through eight goats covering five hundred and sixteen days, were made. In calves a passage through three calves, three hundred and eighty-one days, and another through three calves for three hundred and seventy-five days showed no change. They also report one immunizing experiment in which residence in a calf of a human virus for one year and seven months showed no change. Baldwin had a similar result in a single calf in which after nineteen months the bacilli recovered remained characteristically human.

* She quotes the results of Kleine's examination of seven cultures from like injuries, two of which failed to cause generalized tuberculosis in a calf. His cultures, however, were of the bovine type culturally and other factors may have been the cause of this failure, loss of virulence in culture, for instance. Only one animal was used and sufficient controls are not given.

Another passage was made with a culture of the human type from the spleen of a five-and-one-half-year-old child. A culture from the mesenteric node of the same child gave a culture of the bovine type. This had been interpreted by some as evidence of change of type in the body of the child. If this were so, one would think that it would be possible to change the culture back to the bovine type by passage through calves. A passage through four calves, however, covering six hundred and eighty-five days, showed no change.

The workers in the *Gesundheitsamt* give examples of spontaneous tuberculosis in their calves. Why should not these be considered evidence of successful passage experiments, they ask. It is true that cultures of two different types were isolated, and this was so in a calf receiving an avian culture. Certainly a pertinent question, especially when one considers that even where others have used a large series of animals, one animal raises our suspicion that a sudden change has taken place, though they failed to show this because of insufficient controls, especially isolations as were done by these workers.

It is impossible to quote further work on this point. The evidence as given is inconclusive as is apparent. Even those who report positive results admit that such change is not invariable even with the same virus. Further, as far as one can see, every successful attempt at variation is a complete change of type, not of virulence alone, and in each case one finds evidence of sudden change. In some cases there is no information as to the original material used.

When we contrast this experimental evidence with the uniformity of results and the ease with which cultures may be classified, the uniform isolation of only the bovine type from cattle and the persistence of these characteristics when the bovine type infects man, we must admit that at least the verdict not proven is justified.

The negative evidence furnished by the persistence of bovine characters in bacilli infecting man is very strong. Apart from the butcher's tubercle, where there is found no change even after eight years, at least, we have the cases of

natural infection. The clinical details give us a reasonable certainty that these cases in many instances have shown the lesions in question over a period of a year or two and even longer. In one case Oehlecker considers that the period was at least six and three-fourths years. These periods of time are as long at least as those found in the passage experiments. If so many cases show this persistence of characters we think that one is justified in assuming that all do, unless proof to the contrary is offered. Unfortunately the experimental method has one inherent error, spontaneous tuberculosis in the animals used, that with every precaution known cannot be ruled out. If it could, the history of the attempts to keep a herd free from tuberculosis would be somewhat different. With all the precautions taken by the different workers to keep their animals separate according to the material inoculated, viz., from cattle or man, means very little. The bovine viruses from man give all the conditions necessary for infection assuming that natural infection was rendered impossible.

Own observations. — We have performed no passage experiments covering more than one animal. Nine strains have, however, been reisolated from calves injected with these strains to test their virulence. The reisolation was through the guinea-pig. The residence in the bovine animal was in each case either one hundred and forty or one hundred and forty-two days. The results of the examination of the cultures are given in tabular form under calf virulence. The cultures were tested with varying completeness, but in no case was there any suggestion of change in any characteristic. Although these results are based on a period of passage covering less than five months and in only one animal, they constitute added evidence that any rapid change of characteristics does not occur. In view of the fact that in the reported positive results one is suspicious that the apparent change was referable to one animal, the negative results of our own work stand in contradiction.

SMITH REACTION.

Resumé. — In 1903 Theobald Smith published his observations on a characteristic difference in reaction curves obtained when the human and the bovine type of tubercle bacillus are grown on glycerine broth. The human type caused an initial drop in the acidity of the medium until it became nearly but not quite neutral, and later increased the acidity until it again approached or slightly exceeded the original reaction. The bovine type rendered the medium less acid, neutral or even alkaline, and although the end reaction for this type was at times higher than the lowest reaction recorded, it never rose to the original level. Later, he published additional observations tending to illustrate the same differences.*

Bang obtained results in the main identical with Smith's. He cultivated a strain of the human type which gave a reaction curve approaching the bovine, but this occurred with one lot of broth only. His bovine viruses were quite typical.

Lewis had one culture which towards the end rendered the glycerin broth alkaline or very slightly acid, though it was otherwise of the human type. One culture of the bovine type isolated from a human source gave a rather high acid end reaction.

Duval reports two cases which he considers atypical. One of his viruses reduced the reaction of the medium to nearly neutral, while the other rendered it very acid. After more than a year of cultivation on artificial media both suddenly changed in character, the first causing the broth to become neutral and then partly acid, the second giving a typical bovine curve.

The Royal Commission finds that the reaction curves can be so grouped as to form a scale of the final reactions with complete gradations from one type to the other. To facilitate observations of the change in reaction of the medium, they also used glycerinated litmus milk and found that all

* See also Journ. Med. Research, xxiii, p. 185.

viruses of the human type which grew vigorously on the milk caused the medium to turn acid and to clot after an initial increase of alkalinity; when the growth was less vigorous, the medium became acid, but failed to coagulate. Poorly growing viruses of the bovine type left the milk alkaline, while those which grew vigorously caused an increase in acidity, but never clotted the milk.

Own observations. — Briefly, our method is as follows: As a culture medium we use unfermented one per cent peptone beef broth which contains five per cent of glycerine and is 1.8 per cent to 2.2 per cent acid to phenolphthalein. On this the viruses are cultivated for several generations and when a good pellicle has been obtained in these preliminary cultures, three flasks (A, B, C) are inoculated with the viruses. Each of the flasks contains thirty-five cubic centimeters of the glycerine broth; making a layer about one and one-half centimeters deep. Titrations are so planned that flask A is tested at the end of fifteen, forty-five, and ninety days, B at the end of thirty, sixty, and ninety days, while C remains unopened until the nintieth day, the end of experiment.

The work has not, until now, progressed much beyond the initial stage; only forty-four viruses having been carried through, each on one lot of broth. Seven of these are of the bovine type. Duplicate tests are being carried out for the entire series.

The bovine viruses have given a typical reaction curve. Of the thirty-seven viruses of the human type thirty-five presented a typical "human" curve, although four have at one time during the experiment gone to or beyond the neutral point. Two viruses, Pulmonary Tuberculosis, No. 551, and Tuberculosis of Joint, No. 558, both isolated from human sources and of the human type, gave a typical "bovine" curve, which did not agree with their general cultural characteristics nor the virulence tests. Both were alike in forming a thin pellicle on the medium. On the whole, however, the weight of the final growth does not appear

to have any constant relation to the amount of acid formed. The final reaction in each of the three flasks inoculated with one strain rarely varied more than .1 per cent to .2 per cent, while there was at times much difference in the weight of the dried pellicles. The seven bovine viruses grew vigorously and the final weight was equal to that of the average growth of the human type.

No initial alkaline stage was observed in four of the thirty-two viruses grown on glycerine milk. All of the human type later became acid, but fourteen had not coagulated the milk at the end of ninety-four days, while the remaining eighteen clotted it in from forty-seven to one hundred and twenty-two days; the degree of firmness of the coagulum was in some instances influenced by excessive evaporation and, therefore, the results need further verification. One bovine virus has remained alkaline and the same is true of virus Tuberculosis of Joint No. 558. Virus Pulmonary Tuberculosis, No. 551, has not been tried on milk.

TABLE OF OBSERVATIONS ON THE REACTION CURVE IN GLYCERINE BOUILLON.

(+ = acid; − = alkaline.)

Diagnosis of Case.	Number and Type.	Reaction recorded after					Remarks.
		15 Days.	30 Days.	45 Days.	60 Days.	90 Days.	
Pulmonary tuberculosis. Adults.	507. H. 1.	1.3 + a	.5 + b	1.5 + a	.8 + b	3.5 + c	
	508. H. 1.	1.0 + a	.2 + b	2.5 + a	2.2 + b	3.1 + c	
	512. H. 1.	1.3 + a	.1 + b	1.5 + a	1 8 + b	3.0 + c	Moderately vigorous growth.
	521. H. 1.	1.2 + a	.5 + b	.7 + a	1.8 + b	2.3 + c	
	522. H. 1.	1.9 + a	1.2 + b	.8 + a	2.6 + b	3.7 + a	Growth on *c* very poor, discarded.
	540. H. 1.	2.0 + a	2.2 + b	.3 + a	.6 + b	2.0 + c	Growth delayed at outset, then vigorous.
	541. H. 1.	.7 + a	2.1 + b	2.3 + a	3.0 + b	2.9 + c	Good growth.
	543. H. 1.	1.0 + a	1.1 + b	2.1 + a	2.7 + b	3.2 + c	Rapid growth at outset, then slow increase.
	549. H. 1.	1.5 + a	.4 b	3.0 a	2.5 b	3.1 + c	Growth on *a* at 45 days much in excess of *b* and *c*.
	551. H. 1.	.2 − a	.5 − b	.4 − d	.2 − d	.3 − c	Growth not vigorous.
	570. H. I.	1.3 + a	.8 + b	2.0 + a	2.8 + b	3.0 + c	
	572. H. 1.	1.4 + a	.8 + b	.8 + a	3.0 + b	3.5 + c	Growth on *a* delayed, later vigorous.

	574.	H. 1.	1.1 + a	.2 + b	1.3 + a	1.8 + b	2.9 + c	
	577.	H. 1.	1.2 + a	.2 + b	1.9 + a	1.9 + b	3.1 + c	
	596.	H. 1.	2.2 + a	1.3 + b	.4 + a	.7 + b	2.4 + b	*c* contaminated.
	599.	H. 1.	.8 + a	Neutral.	1.5 + a	1.6 + b	2.4 + c	Vigorous growth from 15 to 45 days.
	603.	H. 1.	2.1 + a	2.1 + a	.6 + a	3.0 + a	2.5 + c	*b* contaminated, growth on *a* delayed, then active, much evaporation.
	622.	H. 1.	1.7 + a	.8 + b	.9 + a	1.4 + a	2.4 + c	*b* contaminated.
	652.	H. 1.	1.0 + a	.7 + b	2.8 + a	2.5 + b	2.6 + c	
Tuberculous adenitis. Children.	167.	H. 1.	1.4 + a	Neutral.	.5 + a	2.2 + b	2.4 + c	Growth on *b* delayed, then vigorous.
	201.	H. 1.	.9 + a	.1 — b	.8 + a	1.7 + b	2.9 + c	Ordinary growth.
	207.	B. 1.	.6 + a	.6 + b	Neutral.	.2 + a	.2 + a	*b* and *c* contaminated.
	393.	B. 1.	1.0 + a	.3 — b	.5 + a	.4 + b	.6 + c	Ordinary growth.
	394.	H. 1.	1.3 + a	.5 + b	2.3 + a	2.5 + b	3.3 + c	Even increase.
	396.	H. 1.	1.2 + a	.1 + b	.8 + a	.8 + b	2.9 + c	
	605.	H. 1.	1.3 + a	.6 + b	2.1 + a	3.2 + b	3.5 + a and b	*c* contaminated.
	636.	B. 1.	1.8 + a	1.0 + b	.2 — a	Neutral.	.2 — c	
	687.	H. 1.	1.7 + a	1.4 + b	.5 + a	.2 + b	2.6 + c	
	705.	H. 1.	1.8 + a	.6 + b	.5 + a	.6 + b	2.3 + c	Growth delayed.

TABLE OF OBSERVATIONS ON THE REACTION CURVE IN GLYCERINE BOUILLON.— *Continued.*

Diagnosis of Case.	Number and Type.	Reaction recorded after					Remarks.
		15 Days.	30 Days.	45 Days.	60 Days.	90 Days.	
Tuberculous adenitis. Adults.	707. H. 1.	1.1 + a	1.4 + b	.4 + a	1.2 + b	2.2 + c	
General tuberculosis. Children.	62. B. 1.	.1 — a	.3 — b	.2 — a	.7 — b	.4 — c	
	63. B. 1.	1.7 + a	.6 + b	.2 — a	Neutral.	.1 — c	
	634. B. 1.	2.2 + a	.4 + b	.2 — b	.2 — b	.2 — c	No growth on *a* after 30 days.
	679. H. 1.	1.2 + a	.5 + b	1.1 + a	2.5 + b	2.3 + c	
Tubercular meningitis. Children.	671. H. 1.	.6 + a	1.9 + b	1.2 + a	2.6 + a	2.8 + c	Growth vigorous from the outset.
	677. H. 1.	1.2 + a	.8 + b	.7 + a	.7 + a	2.4 + a	*b* and *c* contaminated.
	704. H. 1.	.9 + a	.5 + b	2.7 + a	3.1 + b	3.5 + c	
Tuberculosis of bones and joints.	558. H. 3.	.3 — a	.6 — b	.2 — a	.6 — b	.2 — c	Growth poor but even on three flasks.
	592. H. 1.	.9 + a	.7 + b	.6 + a	1.2 + b	2.5 + c	

	638.	H. 1.	1.2 + a	.4 + b	3.0 + a	1.6 + b	2.8 + a	*c* contaminated.
	662.	H. 1.	1.2 + a	.6 + b	.8 + a	.6 + b	1.3 + a	Growth not vigorous.
Viruses of bovine origin.	491.	B. 1.	1.0 + a	.3 + b	.4 — a	.2 — b	.2 — c	Vigorous growth.
Viruses from milk.	111.	H. 1.	1.7 + a	1.0 + b	.1 — a	Neutral.	2.0 + a and b	Growth on *c* poor, discarded.
Miscellaneous.	661.	H. 2.	1.5 + a	.8 + b	1.8 + a	2.8 + b	3.5 + c	

Correlation of results. — The results we have thus far obtained agree essentially with those of Smith. We have not yet done sufficient work to say how far intermediate reactions occur, such as were observed by the Royal Commission. The findings of Lewis and of the Royal Commission would seem to indicate that we are not dealing with a marked biological difference. This is especially true of Lewis's observations, which form true exceptions rather than transitions from one type to another. Duval's results are unique and require corroboration. If these various exceptions, as well as those noticed by Bang and by ourselves, are constant for a particular strain, the value of the reaction is lessened. If uniform and constant differences can be shown to exist in the great majority of cases between the reaction curves of the two types, the test would be a valuable corroborative evidence, although its practical usefulness is hampered by the time necessary to obtain the final reactions, especially for the bovine viruses.

CONCLUSIONS.

Tubercle bacilli as isolated from man fall into two groups. One of these groups is identical in all its characters with that found in cattle. That is, all tubercle bacilli from man and cattle fall into two groups which have been designated the human and bovine types.

Each type shows certain differences, the most important for separation being those found culturally and in virulence. The great majority of cultures group themselves around two extremes, from which there are a few cultures showing variant characteristics. There is no overlapping of characteristics.

These two types are probably different because of residence in different hosts over long periods of time and as such are stable. The evidence in favor of rapid change of type is incomplete and inconclusive.

TABULAR SUMMARIES OF CASES EXAMINED.

It is evident on reading the tabulation of cases as given by individual authors, or those attempting to summarize the

results up to date, that a tabulation founded on some uniform method would be of value. We have, therefore, analyzed each individual case and attempted to formulate a set of diagnoses covering clinical or anatomical details. The following headings were finally decided upon and, although open to criticism, they have the advantage that even if defective the tabulations are comparable.

As it stands, there is difficulty at times in deciding where a case should be placed, but the majority of cases fall easily into one group or the other. Certain divisions are not mutually exclusive because in one instance an anatomical diagnosis is used, in another the clinical diagnosis, which with autopsy could have fallen under a different heading. It seemed, however, advisable to attempt to show the extent of the process where possible.

1. Pulmonary tuberculosis. — Where the lesions are confined to the thoracic organs, clinical or post-mortem diagnosis. A few lesions elsewhere disregarded, especially where abdominal lesions were present which might be regarded as infection by ingestion rather than true generalization.
2. Tuberculous adenitis. — Other than cervical or internal nodes.
3. Tuberculous adenitis. — Cervical. Where the nodes are apparently only infected or this lesion is overwhelmingly predominant. Where this lesion was primary as far as could be ascertained. If abdominal lesion also, is classified under that heading. If part of a generalized tuberculosis is classified under that heading.
4. Abdominal tuberculosis. — Tuberculosis of intestines, abdominal lymph nodes or peritoneum, with or without tubercles in the spleen or liver. There may be coincident lesions in the cervical nodes. If there was spreading to thoracic organs see generalized tuberculosis.
5. Generalized tuberculosis. — Alimentary origin.
6. Same, with involvement of meninges (Cases with cultures from the meninges are noted).
7. Generalized tuberculosis. — Respiratory or unknown origin.
8. Same, with involvement of meninges.
9. Tubercular meningitis. — Clinical diagnosis, extent of generalization unknown, or associated with localized lesions elsewhere.
10. Tuberculosis of bones and joints. — Where these lesions are the essential part of the disease.
11. Tuberculosis of genito-urinary organs.
12. Tuberculosis of skin.
13. Miscellaneous cases.

Subdivisions 3, 4, 5, and 6 will then contain all cases of alimentary infection as far as can be determined from the statements of the different investigators.

This classification will refer mainly to the distribution. In the children it seemed advisable to give some idea also of the severity of the disease. In the cases, therefore, of abdominal and generalized tuberculosis exclusive of the meningeal cases which are fatal, we have subdivided the cases in the children according to the severity of the disease. This has been done by splitting the space for these cases by a vertical ruling and placing the serious or fatal cases to the left of the line, and the cases with slight or accidentally discovered lesion to the right of the line. This has not been done with adult cases, so that slight or latent lesions which at times were disseminated are included. These are few in number and it seemed that to further complicate the tables by separating these cases would give no commensurate gain. Where a bovine infection, however, is noted in an adult, the extent and severity of the lesion will be given.

Absolute completeness is not affirmed for the following table, but it is complete in that it covers all the important series of cases. It would have been possible in reviewing the literature covering inoculation experiments to pick out cases for tabulation or summary, but in most instances the details would be found insufficient and the result not worth the labor involved. Other reports have been found, but were unavailable.

The tables include only cases where pure cultures were isolated. In the text are given cases which either were part of a series or seemed important enough for mention.

The final tabulation is a composite of the tables only. For economy of space the different authors are given in one table. Where the work is from one laboratory or group of workers, the figures are not given separately according to authors. The name of each author in the composite tables is given a small letter and his cases given in the table on the line marked by this letter.

TABULATION OF CASES REPORTED BY

a. Smith; Smith and Brown; and Lewis.
b. Ravenel.*
c. De Schweinitz, Dorset and Schroeder; and Mohler and Washburn.*
d. Kossel, Weber and Heuss; Weber and Taute; Oehlecker and Dieterlen.
e. Weber.
f. Hoelzinger.
g. De Jong-Stuurmann.
h. Dammann and Müssemaier.*
i. Henschen, Jundell and Svenson.
j. Rabinowitsch;* and Beitzke.*
k. Royal Commission.
l. Watt.
m. Kitasato.
n. Hess.
o. Gorter.
p. Fibiger and Jensen.*
q. Burckhardt.*

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Pulmonary tuberculosis.	a	9*	—	1	—	—	—
	b	2	—	—	—	—	—
	c	13*	—	—	—	—	—
	d*	62	—	1	—	5	—
	g	—	1?*	—	—	—	—
	h	2	—	—	—	—	—
	i	1	—	1	—	—	—
	j	8	—	—	—	2	—
	k	10	—	—	—	—	—
	m	152*	—	—	—	—	—
	o	21*	—	—	—	—	—
	p	10	—	—	—	—	—
Totals . . .	. . .	290	1?*	3	—	7	—
Tuberculous adenitis. Axillary.	d	—	—	—	—	1	—
	i	—	—	—	—	1	—
	p	1	—	—	—	—	—
Totals . . .	. . .	1	—	—	—	2	—

* Where figures, names or letters are starred, see notes under author's name following this tabulation (page 141).

TABULATION OF CASES REPORTED. — *Continued.*

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Tuberculous adenitis. Cervical.	a*	5	1	2	7	—	3
	c	—	—	2	—	—	—
	d	2	—	4	3	6	3
	h	1	—	—	—	—	—
	i	1	—	1	—	1	—
	j	4	—	1	—	1	1
	k*	—	—	4	2	1	1
Totals . . .	. . .	13	1	14	12	9	8
Abdominal tuberculosis.	c	—	—	— —	1 —	— —	— —
	d	6	—	— 2	2 2	1 2	— 4
	h	1	1*	— —	— —	— —	— —
	j	2	2	— 4	— 1	— 1	1 —
	k	—	—	— —	— —	— —	3 —
	n	1	—	— —	— —	2 —	— —
	p	—	—	— —	— —	— —	1 —
	q	4	—	— —	— —	— —	1 —
Totals . . .	. . .	14	3	— 6	3 3	3 3	6 4
Generalized tuberculosis. Alimentary origin.	a	2	—	— —	— —	1 —	1 —
	b	—	—	— —	— —	1 —	— —
	c	—	—	— —	1 —	— —	1 —
	d	3	—	1 —	2 —	— —	— —
	f	—	1*	— —	— —	— —	— —
	h	—	—	— —	— —	2 —	— —
	j	1	—	— —	— —	2 —	1 —
	k	—	—	1 —	— —	6 —	4 —
	p	—	—	— —	— —	— —	2 —
Totals . . .	. . .	6	1	2 —	3 —	12 —	9 —

* Where figures, names or letters are starred, see notes under author's name following this tabulation.

TABULATION OF CASES REPORTED. — *Continued.*

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Generalized tuberculosis including meninges. Alimentary origin.	a	—	—	—	—	1	1
	b	—	—	—	—	—	1
	d	—	—	1	—	—	3
	j	—	—	—	—	1	—
	k	—	—	—	—	1	3
Totals . . .	. . .	—	—	1	—	3	8

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Generalized tuberculosis.	a	1	—	—	—	—	—
	c	1	—	1	—	—	—
	d	15	—	—	1	2	—
	h	3	—	1	—	1	—
	i	1	—	—	—	—	—
	j*	4	—	1	—	8	2
	k	1	—	—	—	1	—
	p	—	—	—	—	2	—
Totals . . .	. . .	26	—	3	—	14	2

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Generalized tuberculosis including meninges.	b	—	—	—	—	1	—
	c	1	—	—	—	1	—
	d	—	—	1	—	9	—
	h	1	—	—	—	—	—
	i	—	—	1	—	—	—
	j	2	—	3	—	14	—
	k	—	—	2	—	1	—
	p	—	—	—	—	1	—
Totals . . .	. . .	4	—	7	—	27	—

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Tubercular meningitis.	c	—	—	1	—	—	—
	l	—	—	—	—	—	1*
Totals . . .	. . .	—	—	1	—	—	1

* Where figures, names or letters are starred, see notes under author's name following this tabulation.

TABULATION OF CASES REPORTED. — *Concluded.*

Diagnosis of Cases Examined.	Authors.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.	
		Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Tuberculosis of bones and joints.	d*	12	—	11	1	13	—
	i	1	—	1	—	1	—
	k	4	—	4	—	1	—
	q	—	1*	—	—	—	—
Totals . . .	. . .	17	1	16	1	15	—
Genito-urinary tuberculosis.	a	1	—	—	—	—	—
	c	1	—	—	—	—	—
	d	2	—	—	—	—	—
	i	2	—	—	—	—	—
	k	2	—	—	—	—	—
Totals . . .	. . .	8	—	—	—	—	—
Tuberculosis of skin.	d	1	—	1	—	—	—
	i	—	—	—	—	1	—
Totals . . .	. . .	1	—	1	—	1	—
Miscellaneous Cases.							
1. Tuberculosis of tonsils.	n	—	—	—	1	—	—
2. Primary tuberculosis of mouth. Cervical adenitis.	e	—	1*	—	—	—	—
3. Tuberculous sinus.	c	1	—	—	—	—	—
4. Sepsis. Latent bacilli.	p	—	—	—	—	1	—

*Where figures, names or letters are starred, see notes under author's name following this tabulation.

MIXED OR DOUBLE INFECTIONS. BOTH TYPES (d).

Generalized tuberculosis. Alimentary origin.	30 years.	Mesenteric node = B. and H. type. Bronchial node = H. type.
	5½ years.	Spleen = H. type. Mesenteric node = B. type.
Generalized tuberculosis including meninges. Alimentary origin.	4 years.	Meninges = H. type. Bronchial node = H. type. Mesenteric nodes = B. type.

Notes to preceding tabulation:

a — Smith. Sputum 1, not included, from the characteristics of this culture as described, it is doubtful whether this was a true tubercle bacillus.

a — Lewis. Dr. Lewis supplied us with the ages of his individual cases of cervical adenitis. One case, age not given. B. type not included.

b — Ravenel. Other cultures than those given, spoken of by author but no details of cases and therefore not available for tabulation.

c — Mohler and Washburn. Sputum c, not included. This culture showed a higher virulence than usual, results too irregular to make any decision. Besides the cases given, they examined cultures from the mesenteric lymph nodes of five children affected with infantile tuberculosis, one of which was the bovine type. No age or detail of cases given.

The advisability of tabulating the cases of these authors seemed doubtful. All the cultures were not fully tested, only representatives of different groups. Great irregularities were present, and although they look upon these as evidence of variability it is more probable that the greater part was due to their methods. The bovine types stand out prominently and comparison with these gives practical certainty that the other cultures were of the human type.

d — Kossel, Weber and Heuss, Weber and Taute. Two of their cases not tabulated. One case of pulmonary tuberculosis, no age stated, gave the human type of bacillus. One case of tuberculosis of bones and joints, child, age not given, was a human infection.

e — Weber. The case given was a woman twenty-nine years old, drank raw milk from a badly tuberculous cow over a period of two years. The condition in the mouth was progressive and the cervical nodes were involved. Râles developed in the lungs and the patient died. No autopsy. Cultures from cervical nodes.

f — Hoelzinger. Case given was twenty-four years old. Abdominal pain since childhood. The disease was progressive and fatal, cultures from lung and mesenteric node. Unfortunately the material first passed through rabbits. Although probably bovine only, a possible mixed infection might have been eliminated in this way.

g — De Jong-Stuurman. Milk maid, twenty-seven years old. Had frequent diarrhea. Undoubtedly the authors had a bovine virus. Whether there was some admixture of the human type one cannot determine from the data given. As the isolation was not repeated, collecting the sputum with all necessary precautions against contamination and at frequent intervals, one cannot say that the lesions were due to the bacillus isolated. One of their calves, presumably, was spontaneously infected from a calf inoculated with this virus.

h — Dammann and Müssemeier. The case of abdominal tuberculosis giving the bovine type of bacillus was twenty-five years of age. Operation was followed by recovery. No ascites five months later. The results of their examinations culturally and in rabbits are practically typical. The inoculation results in calves and swine contradict each other and are not parallel with those in rabbits. For a complete criticism of their work see Kossel, Cent. f. Bakt. Orig., vol. 42, pp. 40 and 489.

j — Rabinowitsch. We have placed the atypical cultures reported in her earlier article in the human group. She says: "The six atypical strains which show deviation from the human type cannot be characterized as bovine strains." The atypical cultures reported in the paper on latent tuberculosis are not included, no details were given on which to base an opinion, and the five so classified had to be omitted.

An additional case of generalized tuberculosis (age, twenty years) given as infection with the avian type. Cultures from the spleen.

j — Beitzke. What was said above concerning "atypical" strains holds true here.

k — Royal Commission. One case (no age given) of cervical adenitis gave the human type of bacillus.

l — Watt. History of case interesting. Farm servant had three children, aged nine, six, and five years. Moved to present farm May, 1906. Oldest child died January, 1907, with symptoms of tubercular meningitis. The youngest died March 18, 1907, with similar symptoms. Autopsy revealed tubercular meningitis and a caseous mesenteric node.

The milk given these children was from a tuberculous cow with udder disease. The milk yielded tubercle bacilli on subsequent examination which were isolated in culture. Cultures were isolated from the cerebrospinal fluid of the youngest child and were of the bovine type. (For reports of similar investigation on infection following ingestion of tuberculous milk, see Zwick and Weber, page 152).

m — Kitasato. Cases reported were from fifteen to sixty-three years. Because of the absence of individual ages and slight discrepancy the limit for children being given as sixteen years, we have included these cases among the adults.

o — Gorter. In a few protocols age not given. Descriptions give a reasonable certainty that they were all adults.

p — Fibiger and Jensen. They give no tabular summary of their results according to type, so that the division into types is wholly our interpretation of their findings. They had strains which showed variations and which are included in our tabulation though we were doubtful about including Case 5. Although the authors admit that high virulence points to bovine origin, they do not admit the reverse and make no division into types.

q — Burckhardt. The cases given are from a preliminary report. Six cases only had sufficient information to be admitted. Twenty-eight other cases are mentioned, the final report is to cover forty-nine cases of surgical tuberculosis. The bovine joint infection was of the knee and ran a favorable course.

TOTAL SUMMARY OF TABULATED CASES.

Diagnosis of Cases Examined.	Adults 16 Years and Over.		Children 5 Years to 16 Years.		Children Under 5 Years.	
	Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Pulmonary tuberculosis . .	290	1?	3	—	7	—
Tuberculous adenitis. Axillary	1	—	—	—	2	—
Tuberculous Adenitis. Cervical	13	1	14	12	9	8
Abdominal tuberculosis .	14	3	— | 6	3 | 3	3 | 3	6 | 4
Generalized tuberculosis. Alimentary origin . . .	6	1	2 | —	3 | —	12 | —	9 | —
Generalized tuberculosis .	26	—	3 | —	1 | —	14 | 2	1 | —
Generalized tuberculosis including meninges. Alimentary origin . . .	—	—	1	—	3	8
Generalized tuberculosis including meninges . . .	4	—	7	—	27	—
Tubercular meningitis . .	—	—	1	—	—	1
Tuberculosis of bones and joints	17	1	16	1	15	—
Genito-urinary tuberculosis	8	—	—	—	—	—
Tuberculosis of skin . . .	1	—	1	—	1	—
Miscellaneous Cases:						
Tuberculosis of tonsils .	—	—	—	1	—	—
Tuberculosis of mouth and cervical nodes . .	—	1	—	—	—	—
Tuberculous sinus or abscess	1	—	—	—	—	—
Sepsis. Latent bacilli .	—	—	—	—	1	—
Totals	381	8	54	24	99	37

Mixed or Double Infections: —3 cases:

Generalized tuberculosis. Alimentary origin. 30 years. Human and bovine type in mesenteric node. Human type in bronchial node.

Generalized tuberculosis. Alimentary origin. 5½ years. Human type in spleen. Bovine type in mesenteric node.

Generalized tuberculosis including meninges. Alimentary origin. 4 years. Human type in meninges and bronchial nodes. Bovine type in mesenteric nodes.

TOTAL CASES—606.

SUMMARY OF OTHER CASES REPORTED.*

DAMMANN AND MÜSSEMEIER:

Pulmonary tuberculosis:

M. 16, 47 years.....Human type.

M. 17, 25 years.....Human type.

Abdominal tuberculosis:

M. 15, 47 years.....Human type.

Generalized tuberculosis:

M. 21, 36 years.....Human type.

ROYAL COMMISSION:

Pulmonary tuberculosis:

H. 2, Sp. A. a.....Both(?) types.

b.....Human type.

Specimen was mixed sputa. Fed to two pigs and two heifers. The isolation of a bovine virus under these circumstances is very weak evidence to our mind that this type was present in the original sputa.

H. 4, 34 years.....Human type.

H. 17, Sp. B.....Human type.

After passage changed to bovine type, the results have already been discussed.

H. 21, 16 years.....Human type.

The bronchial nodes only were tuberculous. Tissue inoculations caused slight lesions in rabbits and calves. No cultures were apparently made from the original guinea-pig. The material from the first calf was passed through two sets of guinea-pigs and a calf. Cultures from the thoracic node of the calf and from the prescapular node through guinea-pigs were eugonic, but virulent. Another culture from the prescapular node through a rabbit was eugonic and avirulent for rabbits.

This case excluded by the Commission because Dr. Cobbett thought there was a possible spontaneous tuberculosis in the calf with resulting mixed culture.

H. 49, 18 years.....Bovine type.

This case given as of alimentary origin because of few stony-hard mesenteric nodes. No cultures were obtained from the lung.

Cultures from the mesenteric node direct showed bovine cultural characteristics.

The virulence tests show a great deal of irregularity. When tested after cultivation for only two months, one calf died of generalized tuberculosis; the other survived and showed only slight dissemination. The dose was fifty milligrams subcutaneously. The rabbits likewise were irregular. Culture isolated from

* Where there is no note, inoculations into animals with tissue emulsions only.

calf showing the fewer lesions was fully virulent, killing the calves, inoculated even in ten milligram doses. On retesting the original cultures when seven months old, ten milligrams were found to be able to cause death in one of the calves. In the very next generation, however, the virus failed to cause generalized tuberculosis in rabbits and when again tested after fourteen months' cultivation was fully avirulent for rabbits and calf.

In this case there is evidence of a great deal of irregularity. Although we are convinced that the virus should be classified as bovine, the fluctuations are so contradictory that it is impossible to account for them. Eastman speaks of the results as paradoxical. That a virus should increase in virulence on cultivation and then lose its virulence is contrary to our ideas. This loss in virulence was noticed in rabbits in one generation. It is impossible to say what was the cause of the irregularity. The final loss of virulence would not be extraordinary, but the initial results can only be explained on the assumption that the culture was in some way deficient. We think that one should reserve judgment in rare instances like this, there being the possibility of variation in conditions beyond our control causing the irregularities.

Generalized tuberculosis:

H. 6, 1 9/12 years .....Human type?

Generalized tuberculosis including meninges:

H. 13, 4 years .....Type?

(For details see passage experiments.)

Tuberculosis of bone:

H. 16, 38 years .....Human type.

(For details see passage experiment.)

Tuberculosis of skin:

H. 53, 14 years.....Bovine type.

This virus was first tested after cultivation for eight and one-half months. While virulent for rabbits its virulence for calves was less than that of other bovine viruses examined by the Commission though higher than that of the human viruses. When retested after fourteen and one-half months' cultivation it had little power of causing progressive lesions in calves, but was still virulent for rabbits. A culture from the first calf inoculated showed no appreciably greater virulence.

Cultures fell in the group of the better growing bovines.

On the whole we are inclined to think that earlier inoculations would have shown greater virulence. The comparison between the calves and rabbits is interesting in relation to the parallelism in virulence between the two. Although this is very complete in early cultures, we have no definite information as to which is lost first. It is fairly definite, however, that if the virulence is lost for rabbits the virulence for cattle is also lost. The reverse is not open to definite conclusions, though it would seem from the above that the rabbit virulence could persist longer.

FIBIGER AND JENSEN:

Abdominal tuberculosis:

No. 4, 19 months.....Bovine type?
Tissue emulsions caused generalized tuberculosis.

No. 6, 93 years.....Human type?

Generalized tuberculosis. Alimentary origin:

No. 13, 5 years.....Human type?
Mesenteric nodes caused generalized tuberculosis in calf, but cultures from this calf even in doses of one hundred and fifty milligrams caused only local tuberculosis. A result like this makes all the other tissue inoculations of very doubtful value.

No. 3, 6 years.....Human type?
Spleen of guinea-pig used to inject a calf, intraperitoneally. Peritonitis with suspicious lesions in the thorax. F. and J. class this as showing virulence.

No. 8, 7 years.....Bovine type?
Moderate generalized tuberculosis in calf.

No. 7, 12 years.....Human type?

No. 2, 25 years.....Human type?

No. 1, 42 years.....Human type?

Generalized tuberculosis:

No. 10, 3½ years.....Human type?

No. 9, 49 years.....?
Mesenteric node injected into calf ten days old. Local lesion *healed.* On autopsy nearest node involved at entrance to thorax. Generalized tuberculosis. The age of the patient, the absence of controls, especially cultures from the original material and virulence tests with them, not to mention the practical impossibility of determining the presence of beginning tuberculosis in a calf of such age, rob the experiment of all value.

Tuberculosis of skin:

No. 14, 46 years.....Bovine type.
Slaughter house tubercle, axillary and supra-clavicular nodes involved, complicated after about five years by
Tuberculosis of lungs.....Human type.
Genito-urinary tuberculosis.....Human type.
Cultures urine and testicle.

F. and J. consider the assumption of two infections as not proven.

EBER:

Pulmonary tuberculosis:

Case 11, 17 years..... .....Human type.

Cavity contents inoculated into guinea-pig, organs * of which were used to inoculate a goat (subcutaneous, neck) and a calf (subcutaneously and intraperitoneally).

Goat, killed six and one-half months, no tuberculosis.

Calf, killed six months, tuberculosis spreading to lymph nodes and over peritoneum to pleura and thoracic nodes. Mesenteric nodes involved. Nothing remaining in the neck except slight lesions in the nodes.

Guinea-pigs were inoculated from the calf and organs used to inoculate a goat subcutaneously and a calf (intraperitoneal and subcutaneous). Goat died sixty days, disseminated tuberculosis, abdominal organs free.

Calf died eighty-three days, generalized tuberculosis.

Cultures from original pigs grew freely, avirulent for rabbits.

Cultures from the second goat and calf *failed.*

Case 14, 45 years. .....Human type.

Lung into guinea-pig, organs used to inoculate calf, (subcutaneous). Killed one hundred and twelve days. Local lesion. Cultures fairly vigorous. (Lost by accident.)†

Case 15, 39 years .....Human type.

Lung into guinea-pig, organs inoculated into calf, (subcutaneous), killed seven and one-half months. No tuberculosis.

Cultures fairly vigorous. (Lost by accident.)

Abdominal tuberculosis:

Case 1, 3 years. Died of scarlatina and diphtheria .....Bovine type.

Intestine into guinea-pig, spleen inoculated into calf (intraperitoneal) which, killed in fifty-two days, showed progressive generalized tuberculosis. No cultures.

Case 5, 4½ years. Died of scarlatina .....?

Mesenteric nodes into guinea-pigs, organs inoculated into two calves (subcutaneous); died thirty-seven days, marked generalized tuberculosis (intraperitoneal); killed one hundred and ninety-seven days, marked tuberculous peritonitis. No cultures.

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* Ground up to form a suspension. † Incubator ran up too high.

Case 6, 8½ years. Died of diphtheria.....?

Mesenteric node into guinea-pig, organs inoculated into calf (intraperitoneal and subcutaneous); killed one hundred and twenty-four days, moderate disseminated tuberculosis (Mittelmässig virulent, Eber). The omentum of this calf inoculated into a second calf (subcutaneous). Died sixty-seven days, marked generalized tuberculosis.

"Somit zweifellos eine Erhöhung seiner Virulence." Eber. Compare with Case 5, where there is even a greater difference in two calves without the necessity of a passage experiment.

Case 7, 5½ years. Died of croupous pneumonia .....Bovine type?

Mesenteric node into guinea-pig, organs used to inoculate calf (intraperitoneal and subcutaneous). Died fifty-one days. Disseminated miliary tuberculosis.

Case 8, 4 years. Failed to cause tuberculosis in guinea-pigs.

Generalized tuberculosis, alimentary origin:

Case 2, 3 months. Death due to ill-usage.....Human type?

Mesenteric node into guinea-pig, spleen inoculated into calf (intraperitoneal); killed sixty-four days. Tuberculous peritonitis, extension to pleura.

Generalized tuberculosis:

Case 3, 5½ years. Died of pneumothorax .....Human type.

Mesenteric node into calf (subcutaneous); killed one hundred and ten days. Local tuberculosis. Mesenteric node also into guinea-pig, spleen inoculated into calf (intraperitoneal); killed one hundred and thirty-two days. No tuberculosis.

Case 10, ½ year.....Bovine type?

Mesenteric nodes into guinea-pig, organs inoculated into calf (subcutaneous and intraperitoneal); died forty-two days. Marked generalized tuberculosis. Cultures from guinea-pig sparse and virulent(?) for rabbits. Rabbits not absolutely conclusive according to details given.

Case 13, 31 years .....Human type?

Spleen into guinea-pig, organs used to inoculate calf (subcutaneous and intraperitoneal); killed seven and one-half months. Tuberculosis of peritoneum, pleura and pericardium, the first negative in guinea-pigs. Cultures comparatively sparse, long forms (lost by accident).

Generalized tuberculosis, involving meninges:

Case 4, 2¼ years .....Human type?

Mesenteric node into calf (subcutaneous); killed one hundred and twelve days. No lesions except thickening on one side of neck.

Case 9, 2½ years ..... Human type

Spleen into guinea-pigs, organs used to inoculate calf (subcutaneous and intraperitoneal); killed nine and one-half months. No local lesions, three tubercles in lungs, separate calcareous nodules in mesenteric nodes. Eber considers this probably a spontaneous tuberculosis contracted from calf of Case 10. Cultures through guinea-pig of mesenteric nodes and spleen were vigorous; virulence in rabbits that of the human type.

Case 16, 4¾ years ..... Human type?

Meninges into guinea-pig, organs inoculated into calf (intraperitoneal and subcutaneous); killed four and one-half months. Local lesion in neck and chronic tuberculous peritonitis.

The peritoneum was inoculated into guinea-pigs, the organs being used to inoculate a second calf in the same manner.

This calf died in thirty days. Generalized tuberculosis.

Rabbits inoculated from this calf failed to develop tuberculosis.

Cultures from original pigs fairly vigorous; culture lost by accident.

Cultures from second calf failed.

It is practically impossible to interpret these findings. A sudden transformation is the least likely explanation and in our estimation an improbable one. Either the first calf harbored some bovine bacilli or the second calf received an overwhelming dose when inoculated on both sides of the neck and intraperitoneally. The contrast between the first vigorous culture and the failure to isolate from the second calf are in favor of the first assumption. The failure of the rabbits inoculated from second calf to develop lesions and the vigorous growth of the first cultures, speaks for the second hypothesis. In our opinion the absence of any exactitude of dose and the multiple inoculations, make this experiment of no value whatsoever.

Case 12, 50 years ..... ?

Pia mater into guinea-pigs, organs inoculated into calf (subcutaneous and intraperitoneal). Died thirty-four days. Marked generalized tuberculosis.

Cultures sparse, later more vigorous, long and short forms equal in number.

Rabbits, intravenous inoculations in one and two milligram doses. Died in twenty-three and twenty-nine days with only miliary tuberculosis of the lungs. Subcutaneous inoculation of ten milligrams caused fatal tuberculosis of lungs and liver, detailed description not given.

The rabbit inoculations, especially those inoculated intravenously, are inconclusive. The age of the case would warrant more conclusive evidence, using more comparative methods of dosage and inoculation before one could accept this as a bovine infection.

Eber has recently published a preliminary report of further work along these lines. Fifteen cases were examined in all, but he gives only partial details of seven cases of pulmonary tuberculosis, aged twenty to fifty-three years.

Culturally, these seven cases showed the characteristics of the human type. In rabbits, except for slight variation, none of which showed any approach to high virulence, the results were typical of the human type. Eber italicizes the fact that these variations were not constant according to virus. He was, therefore, not able to show a scale of virulence according to rabbits. Further, he thinks these variations in the individual rabbits detract markedly from the value of these animals for testing virulence. Four of the cultures were tested on calves in doses of fifty milligrams subcutaneously, or the same dose both subcutaneously and intraperitoneally.

The organs of guinea-pigs receiving the original material were inoculated into calves both subcutaneously and intraperitoneally. The result in three cases was a widespread chronic peritonitis. The local lesion in the neck, however, being circumscribed; in one case besides the local effects in the peritoneum and neck there was tuberculous involvement of mediastinal and portal nodes and a few tubercles in the liver. Inoculations of the original material of the last case subcutaneously in rabbits caused death in one hundred and twenty-nine and one hundred and seventy-six days with generalized tuberculosis. Eber considers this an evidence of greater virulence, but he was injecting impure material and the dose was not measured.

In the two cases causing the most lesions in calves, the peritoneal tubercles were inoculated into guinea-pigs, and calves inoculated with their tuberculous organs as above. Both these calves died in thirty-one and thirty-five days, showing widespread tuberculous peritonitis and acute miliary tuberculosis of the lungs.

Cultures were isolated from the original calves in these two cases and also from the third calf showing tuberculous peritonitis. The cultures were from the peritoneal tubercles. These cultures "resembled" more the bovine than the human type. The inoculations into rabbits caused generalized

tuberculosis. The calves inoculated intraperitoneally and subcutaneously with fifty milligrams of culture all died acutely in twenty-one, twenty-four, and thirty-four days, showing widespread tuberculous peritonitis and acute miliary tuberculosis of the lung.

These three and Cases 6, 11, and 16 make a total of six cases in which Eber attempted to increase the virulence of viruses from human sources with apparent success in each case. This fact alone that he never failed stands in direct contradiction with even the work of others claiming apparent success. Case 6 needs no other criticism than that there were no controls. Case 11 also has no controls, especially cultures from the subcutaneous local lesions of the inoculated animals. Case 16 has many inherent contradictions with accompanying lack of data to interpret them.

The most pertinent irregularity to our minds is the apparent contradiction in Case 5. If such irregularities can arise with methods employed the other results are founded on insufficiently controlled data. The three cases with cultures are given with insufficient details. Whether the controls were sufficient remains to be seen. As these are the only cases with any degree of correlation of methods they are the most important. As the total work now stands there is nothing convincing.

The least convincing fact is the uniformity with which apparent change has taken place. In fact if such change were so rapid and uniform, we wonder how any one has ever been able to isolate a single bovine virus from man.

The following investigations are appended because of their bearing on certain points at issue:

Zwick.*—Two supposed cases of milk infection. Both children drank plentifully of milk from a cow with udder tuberculosis. On finding the cow thus badly infected the tuberculosis in the children was naturally referred to this source and on their death cultures were isolated and tested with the following results. Seven other children are healthy.

* Cultures from these cases and from the cow also reported by Weber and Taute and details also given by Weber.

Generalized tuberculosis including meninges .....Human type.
K. L., 4 years. Pott's disease for one year, ill for about two months. Cultures from meninges, lung and vertebral abscess.

Genito-urinary tuberculosis.....Human type.
Kath. L., 16 years. Primary tuberculosis of uterus and tubes, tuberculous peritonitis. Localized tuberculous nodules in lungs, perforation of small intestine. Cultures from mesocolon, omentum, mesenteric node, lungs and uterus.

These two cases clearly show the absolute necessity of a bacteriological diagnosis of type, and of how little value are other methods in determining type of infection and probable source of infection.

Weber reports that in the period from early in 1905 till April, 1909, one hundred and thirteen instances of udder tuberculosis were reported to the *Gesundheitsamt*. About six hundred and twenty-eight persons partook of the milk, mostly over long periods. In forty-four instances it was stated that the milk was boiled or used mixed with that of many cows. In spite of this and with the close contact with cattle as tuberculous as these, there must have been some transference of the infectious agent. The remaining sixty-nine instances are mainly considered. Milk from these cattle was taken raw or as milk products by three hundred and sixty persons, one hundred and fifty-one being children. In only two instances (ages one and five-sixths years and one and one-fourth years) could definite infections from the milk be determined. They were both cases of cervical adenitis and the bovine type of bacillus was isolated. Both alive and well, two and one-half and one and one-half years later. The other children in the same families, eight in all, ranging in age from three to twelve years, show no evidences of infection.

A small number of cases were tuberculous suspects in whom cervical nodes were enlarged, or isolated cases giving abdominal symptoms. It was not possible to examine these cases bacteriologically. At least none showed any progressive symptoms but rather the reverse. Another series of suspects were proven non-tuberculous. In three instances (one, the cases already reported by Zwick) the cultures gave the

human type of bacillus; in two this was to be expected, the cases being adults.

Interesting contrasts in susceptibility are noted, thus in four instances, two calves and six swine developed tuberculosis from the same milk, which was innocuous for the persons drinking it.

Gaffky inoculated the mesenteric and bronchial nodes from three hundred children into guinea-pigs. The ages varied up to thirteen years. Thirty-three or eleven per cent of these children were tuberculous and three were probably tuberculous. Of these thirty-six, twenty-seven gave positive results in pigs; in seventeen both nodes were positive, in five, mesenteric, and in five, bronchial only, positive. Of the remaining two hundred and sixty-four cases, apparently non-tuberculous, thirty cases gave positive results, twelve in both groups, six in mesenteric, and twelve in bronchial only. Of the three hundred children, then, fifty-seven or nineteen per cent showed tuberculous nodes; twenty-nine in both groups, eleven in mesenteric only, seventeen in bronchial only.

Of the positive cases, fifty-three were infections with the human type. In two, the examination was not complete. In the two remaining cases, cultures had not as yet been successful. These were two bronchial nodes, the mesenteric nodes being negative. The cases are, in all probability, infections of the bovine type.

TABULATION OF OWN CASES.

As has been stated these cases were unselected, that is, all material sent from the different institutions was examined regardless of the type of infection.

Besides the tabulation of cases according to type of infection we give two tables which give the type of infection in the fatal cases examined. The series from the Babies' Hospital is given separately, because they represent average conditions better than the total cases.

The cases from the Foundling Hospital are tabulated because these cases form an unusual series. Although complete details were not obtainable, these cases constitute a set of practically exclusively cows' milk-fed children.

TABULATION OF CASES EXAMINED.

Diagnosis of Cases Examined.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children Under 5 Years.		Notes.
	Human.	Bovine.	Human.	Bovine.	Human.	Bovine.	
Pulmonary tuberculosis.	278	—	8	—	5	—	Clinical diagnosis only known, and therefore no positive details as to extent of tuberculosis elsewhere than in lungs.
Tuberculous adenitis. Inguinal and axillary.	1	—	4	—	—	—	(See next.)
Tuberculous adenitis. Cervical.	9	—	19	8	6	12	In two cases cultures were from axillary nodes but primary focus was cervical. Another case died shortly afterwards with pulmonary tuberculosis.
Abdominal tuberculosis.	1	—	1	1	—	3	Milk supply of one child subsequently examined. Tubercle bacilli isolated.
Generalized tuberculosis. Alimentary origin.	—	—	—	—	1	1	Only two cases are given under this heading. Many of the cases in the following subdivisions showed marked intestinal lesions and some possibly were of alimentary origin.
Generalized tuberculosis.	2	—	1	—	12	4	One bovine case had tuberculous osteomyelitis of metatarsal bone.
Generalized tuberculosis including meninges.	—	—	—	—	18	1	
Tubercular meningitis.	—	—	1	—	14	1	No autopsy. Extent of lesions elsewhere unknown.

TABULATION OF CASES EXAMINED. — *Continued.*

Diagnosis of Cases Examined.	Adults 16 Years Over.		Children 5 to 16 Years.		Children Under 5 Years.		Notes.
	Human.	Bovine.	Human.	Bovine.	Human.	Bovine.	
Tuberculosis of bones and joints.	1	—	10	—	6	—	
Genito-urinary tuberculosis.	3	1	1	—	—	—	The adult bovine case was tuberculosis of one kidney. Removal of kidney. Complete recovery.
Tuberculous abscesses.	1	—	—	—	—	—	Possibly primary in bone.
Totals	296	1	45	9	62	22	

Double infection. Both types isolated. Generalized tuberculosis including meninges, 13 months. Mesenteric nodes gave human type. Meningeal fluid gave bovine type.

TOTAL CASES — 436.

TOTAL FATAL CASES IN CHILDREN.

Diagnosis.	Children 5 to 16 Years.		Children Under 5 Years.		Notes.
	Human.	Bovine.	Human.	Bovine.	
Pulmonary tuberculosis.	—	—	5	—	One case included, probably fatal, data incomplete.
Tuberculous adenitis.	1	—	—	—	Other cases recovered as far as known.
Abdominal tuberculosis.	—	—	—	3	Two other cases, one bovine and one human case were operative with recovery, as far as known.
Generalized tuberculosis.	1	—	13	3	Two other bovine cases died directly of exanthemata with complications.
Generalized tuberculosis including meninges.	—	—	18	2	One case gave both types of bacilli; included under bovine as this type caused the meningitis.
Tubercular meningitis.	1	—	14	1	
Totals . .	. . . . .	. . . . .	50	9	or 15% due to bovine type.

FATAL CASES. BABIES' HOSPITAL.

Diagnosis.	Children 5 to 16 Years.		Children Under 5 Years.		Notes.
	Human.	Bovine.	Human.	Bovine.	
Pulmonary tuberculosis.	—	—	4	—	One case included probably fatal. Information incomplete.
Generalized tuberculosis.	1	—	9	2	
Generalized tuberculosis including meninges.	—	—	15	1	One case gave both types of bacilli, included under bovine, as this type caused the meningitis.
Tubercular meningitis.	—	—	11	1	
Totals . .	. . . . .	. . . . .	39	4	or 9% due to bovine type.

CASES. FOUNDLING HOSPITAL.

Diagnosis.	Children Under 6 Years.*		Notes.
	Human.	Bovine.	
Abdominal tuberculosis.	—	1	
Generalized tuberculosis.	3	3	Two of these cases died directly of exanthemata; the tuberculosis was not altogether negligible. These two cases not included in total fatal cases.
Generalized tuberculosis involving meningitis.	1	1	
Totals	4	5	Total cases, 9.

* Six of these nine cases were under two years, one case over five years.

COMBINED TABULATION, CASES REPORTED AND OWN SERIES OF CASES.

Diagnosis.	Adults 16 Years and Over.		Children 5 to 16 Years.		Children. Under 5 Years.	
	Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Pulmonary tuberculosis . .	568	1?	11	—	12	—
Tuberculous adenitis. Axillary or inguinal . .	2	—	4	—	2	—
Tuberculous adenitis. Cervical	22	1	33	20	15	20
Abdominal tuberculosis . .	15	3	7	7	6	13
Generalized tuberculosis. Alimentary origin . . .	6	1	2	3	13	10
Generalized tuberculosis .	28	—	4	1	28	5
Generalized tuberculosis including meninges. Alimentary origin	—	—	1	—	3	8
Generalized tuberculosis including meninges . . .	4	—	7	—	45	1
Tubercular meningitis . .	—	—	2	—	14	2
Tuberculosis of bones and joints	18	1	26	1	21	—
Genito-urinary tuberculosis	11	1	1	—	—	—
Tuberculosis of skin . . .	1	—	1	—	1	—
Miscellaneous cases:						
Tuberculosis of tonsils .	—	—	—	1	—	—
Tuberculosis of mouth and cervical nodes . .	—	1	—	—	—	—
Tuberculous sinus or abscesses	2	—	—	—	—	—
Sepsis. Latent bacilli .	—	—	—	—	1	—
Totals	677	9	99	33	161	59

Mixed or double infections, 4 cases.

TOTAL CASES — 1042.

It is evident from the data given in the cases of mixed infection that the cases reported as generalized tuberculosis including the meninges show a possible source of error unless the meningeal fluid was examined as well as the lesions from the internal organs. That is, the meningitis may be due to a different type of organism than the other lesions. This possible error would be very small and for this reason we have given the cases in the tables whether cultures were examined or not. Any one wishing more specific information can compare the following tabulation with those already given. In the same way the information is not complete if the culture is only from the meningeal fluid. This, however, is negligible, as we are mainly interested in the cause of death, not the possibility of a less serious infection elsewhere.

GENERALIZED TUBERCULOSIS INCLUDING MENINGES.

	Children 5 to 15 Years.				Children Under 5 Years.			
	Human.		Bovine.		Human.		Bovine.	
	Culture.	No Culture.	Culture.	No Culture.	Culture.	No Culture.	Culture.	No Culture.
Alimentary origin:								
Reported cases	—	1	—	—	1	2	2	6
Own cases	—	—	—	—	—	—	—	—
Origin respiratory, or unknown:								
Reported cases	2	5	—	—	9	18	—	—
Own cases	—	—	—	—	13	5	1	—
Totals	2	6	—	—	23	25	3	6

NOTE. — Since the completion of this article a report of three cases of bovine infection has appeared, which we append because one of the cases is a bovine infection in an adult:

Abdominal tuberculosis. 20 years ..... Bovine type.
Died of epilepsy; calcified mesenteric node only manifest tuberculous lesion.

Generalized tuberculosis. Alimentary origin. Infant..... Bovine type.

Tubercular meningitis. 17 months ..... Bovine type.
Cultures from meningeal fluid; mesenteric nodes enlarged; no focal lesions; tubercle bacilli in smears.

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CITY OF NEW YORK

DR. WILLIAM H. PARK, DIRECTOR

VOLUME VI

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DEPARTMENT OF HEALTH

OF THE

CITY OF NEW YORK

ERNST J. LEDERLE, Ph.D.
Commissioner of Health

DR. HERMANN M. BIGGS
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Secretary

PREFACE TO VOLUME VI

The work of the Research Laboratory for 1911 is partly published in the Annual Report of the Department of Health, and partly in this volume of Laboratory Studies. The statistical matter, as in previous years, will be found in the first-mentioned volume, while here is included only the reports of the original investigations.

The recipients of this volume will confer a favor on their colleagues of the Research Laboratory by sending their publications in exchange. These should be addressed to the Librarian Research Laboratory, Foot of East Sixteenth Street, New York.

W. H. P.

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ON A NEW METHOD FOR CONTROLLING THE ADMINISTRATION OF SERUM IN EPIDEMIC MENINGITIS

(Preliminary Note)

Abraham Sophian, M. D.

At the present writing I am compiling my records of about 200 cases of meningitis, in which in all about 600 lumbar punctures were done. Most of the cases were studied in the present epidemic in Dallas, Texas. I thought it would be desirable, before waiting for the compilation of the complete records, to write a preliminary note on an improvement in the method of administering intraspinally the antimeningitis serum, which I have been using for the past seven months, and which I believe has now definitely proved its efficiency and superiority over the old method.

The present method of standardizing the antimeningitis serum, as generally accepted, is by determining the opsonic index and by similar immune serum tests. There is, however, no measure of efficiency placed on the serum as we have in the case of diphtheria or tetanus antitoxin; the dose is therefore measured entirely by volume. Since the serum acts principally by its local action, the idea was, and still is, to introduce as much serum as possible without injury. It was believed that after removal of a quantity of cerebro-spinal fluid by lumbar puncture that one could, to a certain extent, reëstablish conditions in the central nervous system by injecting an equal or slightly smaller volume of serum. Thus in an ordinary, moderately severe case in an adult, if one withdrew 45 c. c. of cerebro-spinal fluid, an injection of 30 c. c. of serum would be safe and ample, while in a severe case one would be justified in injecting 45 c. c. or more of serum.

It is seen by analyzing this method that one hopes and believes that conditions are reëstablished, but one has no proof. On this assumption, many severe symptoms occurring either during or a few hours after an injection had been attributed to the disease proper. Undoubtedly toxæmia, in some cases, accounts for the symptoms; an analysis, however, of many cases would make one suspicious that possibly the symptoms in some were the direct result of the injection, especially when they occurred immediately during or after the operation.

I had been dissatisfied with the old method for a long time. There was no reliable guide indicating the quantity of serum one could safely inject. It occurred to me that if one could make determinations of the cerebro-spinal fluid pressure during the operation one might so have a definite guide, the idea being to inject serum only in sufficient quantity to bring the cerebro-spinal fluid pressure to the original reading before the removal of fluid. In that way one would cause least disturbance or shock. To obtain such result was evidently the reason for instituting the old arbitrary method. I therefore began to take cerebro-spinal fluid pressure readings during the puncture; after withdrawing a quantity of fluid, which reduced the pressure to normal, I attempted to inject serum in sufficient quantity to raise the pressure to the original reading before the removal of fluid. Thus, if the initial cerebro-spinal fluid pressure reading was 400 mm. (water pressure) I removed the fluid till the pressure dropped to normal, or below, usually to between 20 to 100 mm. I then attempted to inject serum in sufficient quantity to raise the pressure to about 400 mm. I shall not cite cases in this paper; the results by this method, however, were misleading and unreliable. Theoretically one would expect a return to the original pressure on injecting a volume of serum equal to the cerebro-spinal fluid withdrawn; this was not the case. One could inject even larger quantities of serum than fluid withdrawn, but the pressure readings were frequently lower than the initial reading before the removal of fluid. Even with readings showing a lower intracranial tension after

the injection of serum, one would meet severe, even very dangerous, symptoms. I learned very quickly that this method was misleading and even dangerous. I believe the error was very probably mechanical; that the later readings were not a true index of the intracranial tension. It is very probable that injecting serum, even with little pressure, would produce a local distention of the subarachnoid space at the site of injection and give readings which did not make a true picture of the uniform intracranial pressure.

I now began to study blood pressure during lumbar puncture, especially with reference to the effect on injection of serum. I have now made blood pressure readings in about 200 cases of meningitis, making from 5 to 700 lumbar punctures. I have found almost uniform results in blood pressure change on injecting serum, and have applied those changes as a guide to the quantity of serum that could safely be injected. Blood pressure change was also frequently a guide, indicating the quantity of fluid that could be withdrawn safely. In a later paper I shall refer in detail to Robinson's[1] work on Blood Pressure in Meningitis, and to Cushing's[2] Studies on the Relationship of Blood Pressure to Change in Intracranial Pressure.

The effect on the blood pressure is inconstant on withdrawing fluid by lumbar puncture in meningitis; most often there is a drop in blood pressure, occasionally quite large, especially on withdrawal of large quantities of fluid. My ordinary procedure in an adult case beginning with an average blood pressure of 110 mm. of mercury is to stop the further withdrawal of fluid if there is a moderate drop in blood pressure, for example, of 10 mm. of mercury; in children of 5 mm.; occasionally the blood pressure begins to drop very quickly as soon as the removal of fluid is begun; the blood pressure then is a guide indicating how rapidly or slowly the fluid may be withdrawn. In other cases there is no change in blood pressure, or even a rise on removing fluid; in these cases one can withdraw as much fluid as possible, usually till the cerebro-spinal fluid pressure is normal, this being roughly measured by counting the drops of fluid as they flow from the needle; one drop of fluid every 3 to 5 seconds corresponds roughly to a normal pressure.

After withdrawal of a suitable quantity of cerebro-spinal fluid the serum is ready to be injected. As usual, the serum is warmed to body temperature, and then injected slowly, either by the gravity method or by syringe. I much prefer the gravity method of injecting the serum; it has many advantages, and few of the disadvantages of the syringe method. As a rule, as soon as the injection of serum is begun the blood pressure drops and continues to drop steadily; reasoning by the old method of injecting serum, one would expect a rise in blood pressure; this, however, is rarely the case. As stated, in the great majority of cases when the injections of serum into the subarachnoid space is begun, the blood pressure drops, and continues dropping steadily as the larger quantity of serum is injected; after there has been a material drop, for example, of 20 to 30 mm. of mercury, the blood pressure begins to drop relatively much faster if more serum is injected. Thus, if an injection of 15 c. c. of serum cause a drop of 20 mm. of mercury in blood pressure, injecting only a few more cubic centimeters of serum may cause a sudden drop of 40 more mm. of mercury, making a total drop of 60 mm. or more. In one robust adult whom I treated there was a drop of 30 mm. of mercury after injecting only 12 c. c. of serum; his was a very severe case, and I wished to introduce as much serum as possible; I injected 3 c. c. more, making a total of 15 c. c. of serum; his blood pressure at one bound dropped 30 mm. more, making a total drop of 60 mm. His clinical signs at this time did not indicate shock; pulse was fair, color was good, but breathing was shallow, and a little irregular. I decided, however, to watch him for a time. A few minutes later he suddenly stopped breathing, then his heart stopped. Immediate active treatment, which I shall indi-

[1] Robinson.
[2] Cushing—Johns Hopkins Hospital Bulletin, Vol. XII. 1901. p. 290.

cate later, gave immediate response. It is easy to see how these patients who have had a large drop in blood pressure, with not much other evidence of shock, may thus suddenly succumb either during or after the injection.

My usual technic in the operation of administering serum is to have one assistant take blood pressure readings throughout the whole operation. If the blood pressure drops during the removal of cerebro-spinal fluid, I use it as a guide, indicating when to stop withdrawing fluid. I accept as a rule the arbitrary drop of 10 mm. of mercury. I use the blood pressure as an absolute guide, indicating the quantity of serum that I can safely inject; here it is also a guide as to how quickly the serum may be injected; as a rule, very rapid injection under considerable force will cause a greater drop in blood pressure. By the gravity method the serum is allowed to run in slowly by gravity, the funnel being raised or lowered to regulate the flow. The barrel of a 10 to 15 c. c. antitoxin syringe may be used as the funnel, attached to about 12 inches of rubber tubing, about ¼ inch in diameter. Ten minutes is a good average time to allow for the fluid to run in, though I have frequently taken 20 minutes or longer, especially in cases beginning with a low blood pressure, or when the blood pressure drops quickly. It is much harder to control the rate of injection by the syringe method, and one may accidentally use too much force in injecting. I have found that a total drop of 20 mm. of mercury in an adult with average blood pressure of 110 to 120 mm. of mercury is a safe indication to stop the further injection of serum. Occasionally there is an initial rise in blood pressure after injecting a few cubic centimeters of serum, followed by a subsequent drop as larger quantities of serum are injected. Very rarely the blood pressure shows a material rise after injecting serum.

Since I began using this method the average dose of serum that I have used has been considerably smaller; frequently not more than 15 c.c., averaging 20 to 25 c. c. when the blood pressure has allowed. I have injected larger quantities also, 30 to 40 c. c.; very rarely more. Judging by the good results with smaller doses of serum, I believe it is very rarely necessary or beneficial to inject more than the maximum of 40 c. c. of serum. The method is of great benefit, not only in the average case, but also in the atypical and difficult one; thus in cases with thick plastic exudate, when the fluid will not flow through the needle, it is a beautiful guide, absolutely indicating how much serum one can inject safely under pressure. Similarly in cases with little exudate, in which one introduces a larger quantity of serum than the cerebro-spinal fluid withdrawn. If the fluid be injected into the subarachnoid space under wrong diagnosis, in cases with posterior basic meningitis, the drop in blood pressure will be very large after injecting only a few cubic centimeters. The blood pressure is also a guide in injecting serum directly into the ventricles in cases of posterior basic meningitis.

What clinical symptoms are associated with the drop in blood pressure? In the order of their appearance these are stupor, which deepens more and more as the blood pressure falls. Respiration becomes superficial and irregular, at times deep, stertorous, slow and irregular; with a large drop in blood pressure breathing may stop suddenly. Pulse will often continue good, even with a large drop in blood pressure, and even with marked change in the breathing; the pulse, therefore, is very misleading. The pupils dilate, increasing with the greater drop. Incontinence of feces and urine occurring during a lumbar puncture usually accompanies a drop in blood pressure, and is a warning.

What is the immediate treatment for severe symptoms during or after the injection of serum? As already indicated with a material drop in blood pressure, further injection of serum should be stopped. If, however, the blood pressure suddenly drops very much, possibly accompanied by the above mentioned symptoms, the indication is immediately to remove fluid from the canal; if one uses the gravity method for injecting this is easily done by lowering the funnel; in bad cases in which the breathing stops as much fluid as possible should be removed and active

artificial respiration begun. Adrenalin in large doses by intramuscular injection, and other vaso-motor stimulants, and atropine help. Even with severe symptoms, if these measures be applied immediately and actively, the patient usually responds.

What effect has adrenalin administered by intramuscular injection, before the puncture, in preventing the symptoms mentioned? Adrenalin administered before the puncture will tend to prevent the large drop in blood pressure on injecting serum. I prefer not to use adrenalin in this way, except in cases with initial low blood pressure.

In reference to the use of general anæsthetics during the operation, I shall say here briefly that in my experience general anæsthesia is dangerous and that it should be used only where absolutely necessary in violent patients. During the administration of an anæsthetic the blood pressure is a comforting guide. I have found that giving many patients water through a straw will keep them quiet; even delirious adults will reflexly suck on the straw and quickly become quiet; during the operation the patient will sometimes drink as much as nine or ten glasses of water. It acts especially well with children. I have referred to it as "water-anæsthesia."

I am now tabulating my results since I have used the blood pressure method in treatment. Most of the cases were studied in the present Texas epidemic. The results were extremely good. The general reaction following the injections was usually not as severe as formerly; temperature did not rise as much; the patient was usually more comfortable and general condition better. The dose of serum as stated was much smaller; very young children, about or under one year especially, at times could not bear more than 2 to 3 or 4 c. c. of serum and in a number of cases responded wonderfully well. In 185 cases roughly tabulated the total mortality was 25 per cent., this including many patients who were brought to the hospital in very bad condition. A number were brought in during the very last stages and some were brought in from a distance of one hundred miles; others with pulmonary œdema, who died a few minutes later. Excluding these apparently hopeless cases in which the patients died from a few minutes to 24 hours after admission, the mortality was about 10 per cent. The cases considered hopeless were marked so on the chart immediately on admission.

Most of the meningitis cases studied were in adults; many patients were past middle age; they included negroes and whites. The mortality was highest among negroes; they seemed to offer little resistance, and easily developed broncho-pneumonia. In children the mortality, after exclusion of apparently hopeless cases, was roughly about 5 to 6 per cent., among them a number being below one year of age. There were also about seven cases of posterior basic meningitis in the list.

Conclusions:

1. The old method of serum treatment is inaccurate and sometimes dangerous.
2. Blood pressure change is a very accurate guide to the quantity of serum that can be safely injected; frequently also indicating the quantity of cerebro-spinal fluid that can be withdrawn.
3. The average dose of serum as controlled by blood pressure is smaller than by the old method.
4. Following an injection of serum, controlled by blood pressure, the after effects are usually much less severe.
5. Mortality figures, which will be accurately tabulated later, show unusually good results.

I desire to express my thanks to Dr. A. W. Nash, City Health Officer of Dallas, with whom I studied the cases in the City Hospital, Dallas, for his courtesy in allowing me to use the hospital records, and for his very valuable coöperation in the studies.

STUDIES ON MENINGITIS DURING 1911

Abraham Sophian, M. D., Phebe L. Du Bois, M. D., and Josephine B. Neal, M. D.

July 1, 1910, the Research Laboratory, under Dr. Park's direction, undertook the study and treatment of meningitis cases in the city and the preparation and standardization of antimeningitis serum.

We were notified whenever a case was reported to the Health Department and we communicated at once with the physician in charge, usually making an appointment with him to see the case. Sometimes owing to the distance we only requested a specimen of spinal fluid. A number of out-of-town fluids have been referred to us from Rockefeller Institute. In this way we have seen 111 cases and examined 214 fluids. Of the cases reported as meningitis that we have personally seen 25 were epidemic cerebro-spinal meningitis, 26 were tubercular meningitis, and 11 were purulent meningitis not due to the meningo-coccus, 30 were diagnosed as some condition other than meningitis, *e. g.*, 11 cases of polioencephalitis, 9 pneumonia, etc.; 9 cases were undiagnosed, and 4 showed normal fluids.

Table I shows how unreliable the statistics must be where no bacteriological examination is made. From the clinical findings, only a probable diagnosis can be made of the exciting organism. This is particularly true of the different forms of purulent meningitis. It is sometimes difficult, too, to differentiate between true meningitis and meningismus, particularly when there is no apparent cause for meningismus, *e. g.*, in early pneumonia.

Tuberculous meningitis is the most common form and is most often confused with epidemic cerebro-spinal meningitis. It can be diagnosed clinically in the majority of cases, for the clinical picture is as a rule definite and distinct. The patients are often well nourished and usually under 2 years of age, though it is not an unusual disease in later life. Frequently a family history of tuberculosis can be obtained. The onset is insidious, of 2 weeks or longer duration. The child is apathetic; there is a little fever and occasional vomiting. Younger children frequently have convulsions—older children complain of headache. The mother often calls attention to the symptoms which are most important in making a differential diagnosis. She complains that the child does not seem to recognize her, is stuporous and sleeps most of the time. This condition of apathy, stupor and impaired mentality is characteristic; the children are stupid, expressionless, do not notice their surroundings, and respond very slowly and indifferently to external stimuli. Other signs of importance are marked internal hydrocephalus, indicated by MacEwen's sign in older children and a bulging fontanel in the younger. Convulsions and local palsies, principally facial paralyses—paralysis of the eye muscles—or paralysis of one arm and leg are more common in tuberculous than in other forms of meningitis. The transitory nature of these palsies is particularly worthy of note. A palsy one day will be gone next day and may reappear as suddenly. Rigidity of the neck and Kernig are only moderate as a rule. The reflexes are irregular; at the onset of the disease usually exaggerated, later diminished or absent. Pupils are frequently irregular and may respond sluggishly or not at all to light. The temperature early in the disease may go up to 101° or 102° F. in the evening, but as the disease progresses may go up to 104° or 105° F. and may show the marked intermittency of meningococcic meningitis. The pulse is irregular and slow, deep, sighing (Cheyne Stokes) respiration is frequently present. Demonstration of tubercles in the choroid is, of course, conclusive. Tuberculous meningitis runs an acute course, death usually occurring from two to four weeks after definite symptoms come on.

TABLE I

(A)

OBSERVATION ON 180 CLINICAL CASES

Clinical Diagnoses Reported by Attending Physician	Final Diagnosis Established by Combined Clinical and Laboratory Examination
Epidemic Meningitis..71 Cases	Epidemic Meningitis....27 Tuberculous Meningitis....17 Ac. Suppurative Meningitis, due to: Pneumococcus.... 4 Streptococcus.... 7 Streptococcus mucosus cap.... 1 Influenza.... 1 Unidentified Gram Coccus (no growth). 1 Enteritis.... 1 Broncho-pneumonia.... 2 Cerebral endarteritis.... 1 Intestinal Intoxication.... 2 Delirium Tremens.... 1 Gastro-intestinal Acidosis.... 1 Measles.... 1 Simple Chronic Internal Hydrocephalus.. 1 No Meningitis (undetermined diagnosis).20
Meningitis unclassified....74	Epidemic Meningitis.... 8 Tuberculous Meningitis....10 Poliomyelitis or Polioencephalitis....13 Enteritis.... 3 Broncho-pneumonia....11 Lobar Pneumonia.... 4 Hysteria.... 2 Purpura Hæmorrhagica.... 1 Intestinal Intoxication.... 2 Cerebro-spinal Lues.... 1 Fractured Skull.... 1 Spastic Paraplegia.... 1
Tuberculous Meningitis....28	Tuberculous Meningitis....23 Epidemic Meningitis.... 1 Streptococcus Meningitis.... 2 Undetermined Diagnoses but no Tuberculous Meningitis.... 2
Poliomyelitis or Polioencephalitis.... 7	Poliomyelitis or Polioencephalitis.... 6 Epidemic Meningitis.... 1

TABLE I

(B)—Summary

Total Cases Reported by Attending Physician		Total Cases in Which Final Knowledge Was Obtained by Combined Clinical and Laboratory Examinations	
*Epidemic Meningitis	71	Epidemic Meningitis	37
Meningitis, unclassified	74	Tuberculous Meningitis	50
Tuberculous Meningitis	28	Acute Suppurative Meningitis, due to:	
Poliomyelitis or Polioencephalitis	7	Pneumococcus	4
		Streptococcus	9
		Streptococcus Mucosus	1
		Influenza	1
		Unidentified Gram Coccus (no growth)	1
		Poliomyelitis	8
		Polioencephalitis	11
		Enteritis	4
		Intestinal Intoxication	4
		Broncho-pneumonia	13
		Lobar Pneumonia	4
		Hysteria	2
		Gastro-intestinal Acidosis	1
		Purpura Hæmorrhagica	1
		Measles	1
		Delirium Tremens	1
		Cerebral Endarteritis	1
		Cerebro-spinal Lues	1
		Fracture of Skull	1
		Spastic Paraplegia	1
		Simple Chronic Internal Hydrocephalus	1
		No Meningitis (undetermined diagnoses)	22

Epidemic cerebro-spinal meningitis has a quite definite symptom-complex also. The children on an average are older. The onset is more acute, with rigors, high temperature, prostration, and projectile vomiting. The cerebral condition is often the direct opposite of that in tuberculous meningitis. In place of apathy, indifference, impaired mentality, there are hypersensitiveness, irritability, constant crying and complaining and objection to any disturbance. Mentality, though disturbed by periods of delirium, is often (except in the last stages of the disease) unimpaired for hours. Retraction of the head, tenderness in the back of the neck and Kernig are marked. Reflexes are irregular, often increased early and later diminished or absent. The pupils respond sluggishly or not at all to light. Palsies are often entirely absent. Bulging fontanel or MacEwen is usually marked, but often to a lesser degree than in tuberculous menin-

*Due to Meningococcus.

gitis. The pulse and respiratory changes are not as great except in cases with marked hydrocephalus, usually an advanced stage of the disease. The temperature curve is usually high and intermittent, but continuous high temperatures, the so-called cerebral temperatures, up to 106 or over, are frequent.

From the above description it is apparent that the two conditions of cerebro-spinal and tuberculous meningitis, while frequently easily differentiated, may sometimes so closely resemble each other clinically, especially in the late stages of epidemic or in the early stages of tuberculous meningitis, that it may be impossible to make a clinical differential diagnosis. *E. g.*, a boy, Italian, six years old, had an acute onset with high temperature, rigors, vomiting, delirium, apathy, stupor, stiffness of the neck. When seen on the twelfth day he was extremely apathetic, stuporous and resistant. Orientation was much impaired, but he did respond occasionally. Neck markedly rigid, Kernig marked. Tache marked, frequent flushing of the face, pupils irregular, fixed; left facial palsy. MacEwen marked. Pulse rapid, irregular, respirations deep, sighing, slow, irregular. Temperature, 105° F. Here the history pointed to a cerebro-spinal meningitis, but the marked stupor and apathy, with evidence of extreme internal hydrocephalus, local palsy, and vaso-motor disturbances, were suspicious of tuberculous meningitis; however, the irritability and restlessness, slight response to questions even at the advanced stage of the disease, the marked rigidity of neck and Kernig, made one lean more to the cerebro-spinal diagnosis. Lumbar puncture demonstrated a very severe infection with meningococcus.

The other acute forms of meningitis, as streptococcus pyogenes, streptococcus mucosus capsulatus, pneumococcus, influenza, present almost the same symptom-complex as epidemic meningitis. The history of previous disease, such as otitis media, may be of some help, but the diagnosis can be definitely established only by lumbar puncture and examination of the cerebro-spinal fluid.

Meningismus complicating other acute infections, while sometimes difficult to differentiate from epidemic meningitis, can as a rule be diagnosed. First, the presence of the primary infection must be established; very often broncho-pneumonia or one of the other conditions mentioned in Table 1. The mental symptoms of restlessness and irritability are not accompanied by the anxiety, constant crying and complaining of the usual epidemic meningitis. Unless delirium is present, the patient is usually bright and intelligent, and responds readily to questions. Rigidity of neck is only moderate, can often be relaxed, and while opisthotonos is present, the patient will still be able to turn his head with ease from side to side. Kernig is also only moderate or absent, and the MacEwen sign, or fontanel bulging, may be present to a lesser degree. With the subsidence in temperature and the improvement in the original infection, the meningeal symptoms disappear.

Poliomyelitis very frequently for several days before palsies appear presents meningeal symptoms, as headache, some rigidity of the neck, some Kernig. The patient usually, however, does not present any of the other cerebral symptoms mentioned. He looks bright and comfortable, and does not give the impression that he is suffering from cerebral disease.

Polioencephalitis often resembles tuberculous meningitis clinically; however, here the local palsies, such as hemiplegia, are extensive and permanent. At other times the clinical signs of an acute active meningitis are present; but here also the palsies are striking. Diagnosis can only be absolutely established by lumbar puncture.

A blood count is of some aid in diagnosis. The cerebro-spinal form shows a slightly higher leucocytosis, up to 20,000 or more, and a distinctly higher relative polynucleosis, up to 99 per cent. Poliomyelitis and polioencephalitis in the febrile stage show a slight leucocytosis. Meningismus secondary to another disease, of course, gives a blood count depending on the underlying cause.

Once a clinical diagnosis of meningitis is made, whatever the form,

a lumbar puncture should always be performed. A clinical diagnosis is never certain, and, even with a complete picture of a type of meningitis, errors may be made.

Table II shows the character of cerebro-spinal fluid in the different types of the infection.

A study of the cerebro-spinal fluid, as the above table shows, will, in the case of acute suppurative meningitis, enable one to find the infecting organism at the first examination as a rule. The exceptions are very rarely in the very onset of epidemic cerebro-spinal meningitis, before the organism is free in the cerebro-spinal fluid, or a localized meningitis, as the localized pneumococcus meningitis mentioned by Holt. It is important especially in the meningococcus infection, where a positive culture is desired, to subinoculate the sediment on suitable culture media as soon as possible after withdrawal from the canal. Where we have been unable to obtain the fluid for inoculation within a few hours after withdrawal we have been able to demonstrate the organism in smears, but sometimes no growth has been obtained. The delicate nature of the organism when first obtained, its tendency to rapid autolysis, explains this. The media used by us for routine subinoculation of sediment has been 2 per cent. glucose serum agar, blood agar, neutral to phenolphthalein.

In tuberculous meningitis the bacilli can, in a very large percentage of cases, be found in the cerebro-spinal fluid at some time during the course of the disease, usually the first examination being sufficient to demonstrate the organisms. Where the cerebro-spinal fluid can be set aside immediately to obtain the unbroken fibrin network, the chances for finding the bacilli are very much greater. Most of the fluids sent to us for examination are considerably shaken up, but centrifuging the fluid for an hour or longer, collecting the sediment on a coverslip and staining for tubercle bacilli has enabled us to find the bacilli in most of the cases. Long and patient search is necessary; the bacilli are usually very few and scattered, and sometimes hours may be spent in search before they are found. An important point to remember is that, where the organism is not found during life an examination of the cerebro-spinal fluid obtained post-mortem will often demonstrate the bacilli in clumps in large numbers. Animal inoculation in positive cases where bacilli have not been found produces tuberculosis in four to six weeks.

Cytological examination is extremely important. The increase in the number of cells in inflammatory meningitis is constant. In the suppurative forms of meningitis the preponderance of polynuclear leucocytes is striking. In chronic cerebro-spinal meningitis a relative lymphocytosis is frequently present. In tuberculous meningitis there is an excess of lymphocytes up to 100 per cent. Occasionally, however, a tuberculous fluid, in which bacilli have been found, will be cloudy and show an excess of polynuclear leucocytes up to 90 per cent.

An attempt to classify any particular type of meningitis merely on the number of cells without the demonstration of the infecting organism, as was recommended by Dunn in the case of tuberculous meningitis, would apparently be an uncertain thing to do, especially in view of the very large percentage of cases of meningitis where the infecting organism may be found.

The gross fibrin content of the fluid after standing is a fairly constant guide, there being an increase in meningitis, and very little fibrin in normal fluid. The reduction test with Fehling's solution has been found of no value by us.

Noguchi's globulin test in a large number of fluids we examined was substantiated; in inflammatory conditions a positive reaction being present indicated an increase of the globulin content in the cerebro-spinal fluid.

In the main, our treatment of epidemic meningitis has been carried out along the lines laid out by Flexner, consisting in the spinal administration of the meningitis serum, being guided by the symptoms of the patient and the condition of the cerebro-spinal fluid. The early recogni-

TABLE II

CEREBRO-SPINAL FLUID

	Normal	Meningism	Poliomyelitis Polioencephalitis	Cerebro-spinal Meningitis	Streptococcus, Pneumococcus, Influenza, etc., Meningitis	Tuberculous Meningitis
Color	Clear	Clear	Clear	Cloudy—pus sediment	Cloudy—pus sediment	Clear—white flakes —fibrin network.
Pressure	Low—escapes slowly drop by drop.					
Quantity	Little—few c.c...	(Up to 50 c.c. or more)	(Up to 50 c.c. or more)	(Up to 100 c.c. or more)	(Up to 100 c.c. or more)	(Up to 100 c.c. or more).
Cytology	Few cells, leucocytes and endothelial	Few cellular elements	Cells increased in number (Lymphocytes, 90% or more)	Cells numerous (Polynuclear up to 100%)	Cells numerous (Polynuclear up to 100%)	Cells numerous (Lymphocytes up to 90%).
Bacteriology	Sterile	Sterile	Sterile	Meningococcus	Infecting organism.	Tubercle bacillus.
Albumin, nitric acid test	Faint trace	Trace	Trace.			
Globulin test	Negative	Negative	Positive in early stages.			*

*NOTE—Where tubercle bacilli are not found in spread from the sediment, animal inoculations are made; if case is tuberculous, this will produce tuberculosis within six weeks.

tion of complications, such as posterior basic meningitis with closure of the foramina, cannot be too strongly emphasized.

We send out the following directions with the serum. Antimeningitis serum is a specific immune serum, of therapeutic value only in meningococcus meningitis. It is administered intraspinally only; the subcutaneous or intravenous routes being practically worthless.

After performing a lumbar puncture under careful aseptic precautions in the 3d or 4th lumbar space as much cerebro-spinal fluid is withdrawn as will come easily, usually 40 to 60 c. c., or more. If the fluid is cloudy, inject the serum immediately without waiting for a bacteriological examination, but do not repeat the injection until examination of the fluid shows the presence of the meningococcus. The serum should be warmed gently to the body temperature and injected slowly without pressure into the subarachnoid space. A syringe may be used for injecting the serum, or it may be allowed to run in by gravity through a funnel attached to the needle by means of rubber tubing. The quantity of serum injected should be slightly less than the amount of fluid withdrawn, so as to avoid pressure symptoms. The injections should be repeated arbitrarily each day for the first 3 or 4 days of the disease, the administration later depending on the clinical symptoms, especially temperature and general improvement and the condition of the cerebro-spinal fluid as revealed by bacteriological examination. Usually only 4 to 6 injections are necessary, though sometimes 15 or more may have to be employed.

In cases with very thick exudate which will not flow through the needle gentle irrigation with warm saline will often start the flow. If this measure fails a small quantity of serum may be injected under pressure. This measure, however, is attended by danger of convulsion, collapse, or even death.

With a complication of internal hydrocephalus shutting off the communication of the ventricles with the subarachnoid space, intraventricular injection of serum is advisable.

At first we used the serum sent out by Rockefeller Institute. Later the horses from which that had been prepared were turned over to us. In the meantime we started 4 horses of our own. For about 2 months they were injected at 5 to 7-day intervals with meningococcus autolysate, beginning with 2 c. c. and gradually increasing it until they were getting 10 c. c. This autolysate was prepared in the following manner: 2 to 3 c. c. of normal saline were added to a 24-hour growth of meningococci on a glucose agar slant, to loosen the growth. A Blake bottle containing 30 c. c. agar was subinoculated with this by pouring it over the surface. At first we used 21 cultures—each one representing a different case of meningitis—later the number increased to 35. The cultures on the Blake bottles were allowed to grow from 18 to 24 hours and then loosened by pouring 10 c. c. of saline on the surface of each bottle. The emulsions of all 21 cultures were then mixed, 2 per cent. toluol added and the bacteria allowed to autolyse in the incubator for from 24 to 48 hours. The toluol gradually evaporated, and at the end of that time the autolysate was put in the ice box ready for use. At the end of two months we began to give the horses live cultures, 2 c. c., increasing to 6 and 12 c. c., prepared in the same way as the autolysate up to the point where toluol is put in. Then we alternated live cultures and autolysate, gradually increasing the interval of time between injections and increasing the amounts given until at the end of six months they were getting 35 c. c. of autolysate and 35 c. c. of live culture, alternating fortnights.

From time to time we tested the serum of these horses and when it had reached a titre of 1:5,000 regular bleedings were begun and so timed that from 10 to 12 days intervened between the injection and bleeding.

We have not yet found what we consider an accurate method of standardizing antimeningitis serum. We have tried out testing by the opsonic index after the manner described by Jobling, the complement deviation test, and neutralizing the fatal doses of live culture and autolysate for animals.

In doing the opsonic test we used the technic recommended by Neufield and also used at the Rockefeller Institute. The limit of phagocytosis is determined by the general appearance of the spread. While this method gives a fairly good idea of the degree of immunity, repetition frequently gives uncertain and contradictory readings, even when great care is taken to select an organism which does not show spontaneous phagocytosis. Some experiments which we have not yet completed lead us to believe that the meningococcus family is made up of a number of different strains which differ from one another in the way they react with their own autogenous serum and the sera of other members of the group. In testing a polyvalent serum, therefore, it would be advisable to use a suspension of all the organisms used in the immunization, but this is impossible, because many of the meningococci show marked spontaneous phagocytosis.

The complement deviation tests have given fairly uniform results. Control experiments were made with normal horse serum, anti-tetanic serum, diphtheria antitoxin, and streptococcus serum and they all showed fixation at low dilutions only. A gonococcus serum of high immunity gave as high fixation with meningococcus antigen as did the antimeningitis serum. This would only show that the test was not specific and would interdict its use as a means of diagnosis, but would not invalidate readings obtained with the known materials, meningococci antigen and meningococcic serum. However, with the new method of preparing antigen suggested by Dr. McNeil we hope to have an absolutely specific antigen.

In comparing the readings from opsonic work and complement deviation tests we found that they agreed pretty well—a high opsonic reading corresponding with a high complement fixation reading.

We did a large number of experiments with guinea pigs to test the protective power of anti-meningococcic serum. Most of the tests were done with guinea pigs weighing about 250 grams. We prepared the bacterial emulsion in as nearly the same way as possible for each series of tests to determine the fatal dose and always put in controls. Our finding results agree with Jobling's and many others. The pathogenicity of the organism varies considerably from day to day and the resistance of individual animals varies a good deal, *e. g.*, one animal will live when given twice the dose that proves fatal to another animal of approximately the same size and age. For our purposes testing by means of animals has proved worthless.

From our experience thus far we have concluded that the therapeutic activity of the antimeningitis serum probably depends to a very great extent upon the opsonins and that it is therefore wise in standardizing to make use of the opsonic test. On account of the irregularity in readings, however, and the impossibility of using the mixed strains of meningococci in the test, the limit of immunity as determined by this method is left in doubt. The complement deviation tests have given uniform results and have corresponded as to degree of immunity with the opsonic readings. The readings are sharp and a mixed antigen is used. At the present time it seems to us wisest to use both methods, the opsonic as a control and the "complement fixation" for more accurate results.

COMPARATIVE RESULTS OF THE ANTIRABIC TREATMENT WITH FRESHLY PREPARED VIRUS WITH THAT SENT TO A DISTANCE

D. W. Poor, M. D.

These statistics are based on a series of 2,851 cases treated from May, 1906, to September, 1911. Only those cases are considered in which data was available to show that the infecting animal was evidently rabid. Out of this total 2,108 cases were treated with virus sent from the laboratory and 737 were treated at the laboratory.

The total mortality in the former series (including cases which died during and after the period of observation) was 0.6 of 1 per cent. In the latter series the total mortality was 0.9 of 1 per cent. On the other hand, the mortality percentage occurring onlv after the period of observation (commonly rated as the percentage of failure of treatment) was 0.0028 per cent. in the cases treated at the patients' homes as against 0.0018 in the cases treated at the laboratory.

These figures are probably affected, first, by the somewhat larger proportion of head bites in the cases treated at the laboratory, second, by the possible carelessness of physicians in administering the treatment or in reporting the deaths.

There is still a great difference of opinion as to the relative value of avirulent and virulent vaccine in the immunization against rabies; some institutes advocating the former, some using only the virulent cords of the Pasteur series; others the fresh undried fixed virus; and still others a mixture of avirulent and virulent dried cords, as illustrated by the original Pasteur scheme.

There is no question that strong immunity may be produced by the use of wholly avirulent vaccine, as is shown in a report on this subject in the Laboratory Report for the year 1908.

There is undoubtedly a slight loss in virulence when the emulsion from a virulent cord is kept at room temperature for from twenty-four to thirty-six hours. It does not follow, however, from this, that there is any loss in immunizing properties. If there is any loss, it is certainly not great.

The results of the experimental treatment of animals are shown in the following table. Two series of animals were treated for fourteen

Series	Number of Guinea Pigs Used	Date of Infection	Result	Result in Controls
A. 1.....	9	2½ weeks after treatment.	All remained well.	6 out of 7 died, incubation of 19 days.
B. 1.....	7	2½ weeks after treatment.	One died of rabies with incubation of 12 days.	6 out of 7 died with average incubation of 16 days.
A. 2.....	7	3 months after treatment.	All remained well.	7 out of 8 died with average incubation of 16 days.
B. 2.....	5	3 months after treatment.	All remained well.	4 out of 5 died with average incubation of 15 days.
A. 3.....	8	6 months after treatment.	All remained well.	6 out of 7 died with average incubation of 15 days.
B. 3.....	3	6 months after treatment.	All remained well.	

days. In one the virus was used immediately after its manufacture and in the other it was kept for from three to four days at room temperature. Each series was divided into three groups and the immunity of the animals tested by injecting them in the leg with 1½ c. c. of street virus. The first group was tested 2½ weeks, the second three months and the third six months after the completion of the treatment. The animals treated with the freshly prepared emulsion are marked A. The others B.

The fact that one of the animals treated with the kept virus developed rabies might be thought to show that the protection given was less than with that freshly prepared.

Two points should be considered: First, that this was an extreme test, the virus being kept more than three times as long as that used on patients, and, second, it may have been mere chance that the one death occurred in this lot rather than the other.

THE LATE CAUTERIZATION BY MEANS OF NITRIC ACID OF WOUNDS INFECTED WITH RABIES VIRUS

D. W. Poor, M. D.

Marie in his work on Experimental Rabies collects the experimental evidence on the value of the local treatment of wounds subsequent to their infection with rabies virus. The experiments consisted in the use of caustics and of amputation of the part infected. The results were so confusing as to lead to the hypothesis that the virus may travel by a slow (axis cylinder) and a rapid (lymphatic vessel) route—either or both in any given case. Whatever may be the explanation, the facts remain that sometimes local treatment is successful in preventing infection and sometimes it is not. That this is not due simply to the degree of thoroughness of the cauterization is seen from the fact that the same irregularity occurs after complete amputation of the part.

Cabot in 1898 published an extensive experiment, in which he compared the results of cauterization at the end of 24 hours after infection. Nitric acid, the actual cautery, silver nitrate, and simple mechanical cleansing of the wound were employed. The results showed a marked superiority in favor of the use of nitric acid, with which he saved 76 per cent. of the animals.

The technic was as follows: One c. c. of an emulsion of fixed virus was injected in the leg of the animal in the region of the sciatic nerve. At the end of 24 hours he exposed the sciatic nerve with an incision and freely cauterized the neighboring tissues.

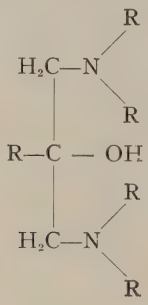
This experiment has been repeated by us with some variation in order to simulate more closely the conditions as they occur in actual clinical practice.

After the removing of the hair with barium sulphide an incision was made in the back of the neck of the guinea pigs and the subcutaneous tissues on either side cut in several places with the scissors. This wound was infected with a swab dipped in an emulsion of street virus and the edges of the wound brought together with adhesive plaster. At the end of 22 hours the wounds in one series of animals were opened up and the tissues treated with another swab dipped in nitric acid. After excluding a number of animals which died from accidental causes, there remained twelve in the series treated with the acid and eight controls. All of the latter died of rabies with an average incubation of 20 days. Five of the treated animals had shown no symptoms of rabies 2 months after infection, at which time they were discharged. The remaining treated animals died of rabies with an average incubation of 23 days. Forty-four per cent. of the treated animals were therefore saved by cauterization with nitric acid at the end of twenty-two hours.

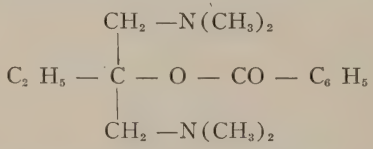
ALYPIN: A VALUABLE MEANS OF ANÆSTHETIZING WOUNDS BEFORE AND AFTER CAUTERIZATION

Paul Bartholow, M. D.

Modern chemistry provides plenty of anodynes, some of which, especially in their anæsthetic power, are not too familiar to medical men. Among them are three alkamine esters, stovaine, novocaine, and alypin, of the type—

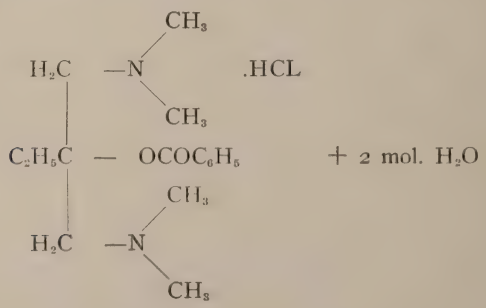


Alypin contains two amino groups, viz.—



It differs from stovaine slightly, which has but one of these amino alcohol groups. It may be described as the dimethyl amino derivative of stovaine.

Because alypin contains these amino groups it is at once a stronger base and a stronger anæsthetic than stovaine. In contrast with stovaine, it has the power to form neutral salts, soluble in water, with a strong affinity for sensitive tissue. The salt that we have used is the monohydrochloride—



It is a fine crystalline substance, which fuses at 169°. It is very soluble in water, easily soluble in alcohol. The solid substance may be

sterilized by heating in the autoclave for 25 minutes; the watery solution by boiling for 5 minutes. A little sodium bicarbonate added to the solution is useful. It keeps it alkaline and increases the anæsthetic effect. Accordingly we have employed a 2 and 4 per cent. solution, as follows—

Alypin—HCL	4.
Distilled water	100.
Listerin, or,	
Liq. antisep.	0.7

Alypin, as Impens[1] discovered, dilates the blood vessels. The addition of adrenalin to solutions is, therefore, of value, *e. g.*—

Alypin—HCL	4.
Distilled water	100.
Adrenaline	1.

What are the physiological effects of alypin? These have been studied with success by Impens and Læwen,[2] who have evaluated the merits of alypin, stovaine, and novocaine with a precision that approaches finality.

Alypin is readily absorbed and excreted. There is no reason to doubt that it is scarcely half as toxic as cocaine. I have not seen any toxic effects when solutions are applied to the skin or to wounds, yet it is absorbed quite easily in this way. The pulse, respiration, and pupils are unaffected. Alypin, indeed, is not mydriatic.

By an ingenious device Impens was able to test its effects on the sensory nerves of the frog. His conclusion is that the anæsthetic action is beyond dispute, being not surpassed by the intense and powerful effects of cocaine. This is high praise. I should imagine from observation that the anodyne effect of alypin is but little inferior to that of cocaine, and certainly superior to that of stovaine or novocaine.

Impens performed his experiments upon frogs and cats. The exact effects of alypin upon the heart and respiration of man are not known. It is hardly worth while to attempt to draw conclusions from the experiments on frogs, but I am not aware of any case of poisoning in humans. It is important to know, as Læwen has shown, that alypin does not injure the substance of motor nerves. Yet it is more powerful than stovaine or novocaine, which do. A few words as to its other merits. It does not injure the tissues. Necrosis does not occur. Its effects are prompt and penetrating.

For very special reasons it seemed desirable to employ such a remedy at the rabies clinic. I suggested the use of alypin to Dr. Poor, who acceded to my plan. The result has been gratifying. Bites, which are common among children, are cauterized with much greater ease, and with much less suffering than formerly. We use fresh solutions, for even alkaline solutions do not retain their power more than seven days, and proceed as follows: A swab of cotton saturated in the alypin solution is laid on the wound a few minutes. The caustic, nitric acid, is then applied, and then the alypin solution again. This simple process is aimed primarily at relieving pain, but it rather helps than hinders the essential thing—the cauterization. For it enables us to apply the caustic more thoroughly than is sometimes possible when we have to deal with a struggling or shrinking patient. The two cases which I attach to this paper are typical of a score of others.

Case 1. Stella F., age 12; bitten on the right arm and on the little and middle finger of the right hand; a number of small, circular bites, which had bled freely. Anæsthetized with 10 per cent. alypin; cauterized with fuming HNO_3.

CASE 2. Helen S., age 3 years and 3 months; bitten and scratched in two places, on the scalp and neck; no bleeding at the time. Bleeding was produced by washing the bites with ether. Anæsthetized with alypin 4 per cent. Cauterized with fuming HNO^3. No pain.

In most cases there was some, though little, pain. The benefit of the anodyne was, however, obvious.

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DIAGNOSIS, PREVENTION AND TREATMENT OF VULVO-VAGINITIS IN CONTAGIOUS DISEASE HOSPITALS

Anna W. Williams, M. D., and M. A. Wilson

The plan of the investigative work on gonorrhœa in the Contagious Disease Hospitals of the New York City Health Department, begun in October, 1910, includes both clinical and laboratory studies. The clinical work was undertaken with the cordial coöperation of Superintendent of Hospitals, Dr. R. J. Wilson, and of the Hospital Staff of Physicians and Nurses. Dr. MacDonald, House Physician of the Scarlet Fever Hospital, Manhattan, has helped materially, not only in keeping clinical records, but in taking cultures and making spreads.

The plan is as follows:

1. *Admission smear and culture.* On entrance examination a smear is made from the vagina after wiping the external genitals with gauze moistened with disinfectant (permanganate of potassium 1:10,000, carbolic acid 2 per cent., or mercuric chloride 1:3,000) and drying with sterile gauze. A culture may also be made on request of physician in charge, or of Director of Laboratories, though this is not necessary for diagnosis if satisfactory smears are obtained.

(a.) *The technic of making smear is as follows:*

Small cotton wire swabs, very tightly wound, are sterilized each in a separate holder. Clean glass slides (sterilized if cultures are to be made from swab) are wrapped each in a separate filter paper.

For obtaining the vaginal material the labia are held well apart by an attendant wearing sterile rubber gloves. The slender cotton swab passes easily into the vagina without touching any external part but entrance. It is rubbed gently about vaginal wall, then withdrawn and *rolled (not rubbed)* quickly over a slide (slide sterilized and held face down if culture is to be made). *In making this smear care is used not to pass the swab over the same surface twice.* In this way a beautifully spread film is made, which we have found much more satisfactory on the whole than the pipette-made film recommended by I. Van Giesen. The swab is then returned to its holder (and covered and numbered if culture is to be made). The air-dried slide is rewrapped in its filter-paper and numbered.

(b.) *The technic for making culture is as follows:*

A small amount of rich, sterile ascitic fluid is added to the tubes containing the swab. (If patient is at a distance from laboratory the ascitic fluid is sent in a separate tube, and is inoculated from swab just after smear is made; then swab is withdrawn from ascitic fluid tube, placed in its own tube, and both tubes are sent immediately to the laboratory.) After being stirred up in this fluid the swab is withdrawn and smeared in strokes radiating from the center over ascitic-agar (1-4) plates containing 2 per cent. glucose. Other plates of the same medium are stroked with platinum loopfuls of the ascitic fluid emulsion. From two to four plates are made and placed in thermostat at 36° C. After 24 hours the plates are examined and if gonococcus-like colonies are seen they are fished. Then a smear is made from the whole of one of the most characteristic streaks, stained by Gram's method and examined for gonococcus-like organisms.

2. *Isolation of case after admission.* The case is kept isolated until report on admission smear is received.

3. *Data for laboratory.* The smear is sent to the laboratory properly labeled, together with a slip containing the following information:

(a) Date of Smear. (b) Name of patient. (c) Age. (d) Disease. (e) Genital discharge and inflammation.

4. *Grouping cases.* From the microscopic examination of *well-made* and *well-stained* smears (stained by Gram's method), and, where necessary, from cultures and from clinical appearance as well, the cases are divided into four groups, as follows:

1. *Positive cases, i. e.,* those showing leucocytes filled with morphologically typical gonococci in smear or showing typical cultures, or showing both.

2. *Suspicious cases, i. e.,* those showing in smears any suspicious intracellular diplococci and 50 per cent. or more of polymorphonuclear leucocytes.

3. *Observation cases, i. e.,* those showing in smears 50 per cent. or over of polymorphonuclear leucocytes, but no suspicious intracellular diplococci; or those having the clinical symptoms of discharge and inflammation and showing less than 50 per cent. of polymorphonuclear leucocytes.

4. *Negative cases, i. e.,* those showing in smears less than 50 per cent. polymorphonuclears, and no suspicious intracellular diplococci, and no clinical evidence of the disease.

5. *Isolation of groups.* Each group is kept isolated.

6. *Later smears.* From the first three groups smears are made once a week until a negative smear is obtained. From negative groups smears are made if any suspicious symptoms appear.

7. *Negative diagnosis from later smears.* Three successive *well-made* and *well-stained* negative smears from first three groups, at intervals of not longer than three days, mean a *negative diagnosis.*

8. *Later cultures.* From cases where morphologically typical gonococci persist in smear, cultures may be made and gonococcus-like organisms isolated and studied to find out if they are true gonococci.

9. *Bathing.* All cases in these groups, unless physician in charge orders otherwise, must be bathed with peculiar care, since this is one of the most important factors in preventing spread. The points to be observed in bathing convalescents are: 1. Bathers must wear rubber gloves, which must be disinfected after bathing genitals of patient, after drying them, and after the whole bath is finished. 2. Put patient on clean sheet over rubber. 3. Soap upper part of the body of patient. 4. Wash external genitals with disinfectant, then with soap and water, using special care to cleanse every part; disinfect cloths used for bathing parts. 5. Soap rest of body. 6. Stand patient when possible in tub and douche with warm water. 7. Dry external genitals last; disinfect towel. 8. Disinfect tub, then wash it with boiling water.

10. *Genital pad.* Each patient in first and second groups must wear constantly a genital pad moistened with solution of permanganate of potash (1:10,000).

11. *Protection of nurses and physicians during care and inspection of patient.* Rubber gloves must be worn by all nurses and physicians when caring for or examining genitals.

By the careful carrying out of the above plan alone without specific treatment the number of apparently positive cases has been reduced to less than 9 per cent.

One of the chief reasons for dividing the cases into four segregated groups was to test the comparative value of the smear and culture methods to its fullest extent. And we were convinced after following 120 cultured cases that the diagnosis from a *well-made* smear is practically just as reliable as that from a careful culture, provided we make the four divisions detailed above. In this study we found that cultures from a certain number of cases, classed as suspicious because the smears showed

a few gram-negative intracellular diplococcus-like organisms, gave in cultures—repeatedly from the same case—either gram-negative coccus forms, or a gram-negative bacillus which shows, besides bacilli in young pure cultures, diplococcus forms very similar to the gonococcus. A very few among the suspicious smears showed gonococci in cultures, while none of the observation or negative smears gave positive cultures. This work, then, is a corroboration of that of others, except that we emphasize more strongly the importance of making no positive diagnosis from a smear unless it gives a clear-cut picture of leucocytes filled with gonococci. A few gram-negative coccus forms in a cell are generally organisms other than the gonococcus. Our results have been corroborated by the clinical course of the cases in each instance.

The statistical tables are given in the yearly report.

Conclusions:

1. All smears showing leucocytes filled with morphologically typical gonococci may be considered positive.
2. Smears showing single and occasional intracellular gram-negative diplococci must never be called positive, since the great majority of them are not. They should be considered suspicious only.
3. Nursing is all important in preventing spread and in helping cure of gonorrhœal vaginitis.

A BRIEF STUDY OF THE EFFECT OF GONOCOCCUS VACCINATION IN GONORRHŒAL VAGINITIS OCCURRING IN CHILDREN CONVALESCENT FROM SCARLET FEVER

J. H. Huddleston, M. D., and A. Sophian, M. D.

The vaccine used was a stock gonococcus vaccine, one strain 9 months old, the others 6 weeks old, a mixture of four strains, each obtained from vaginal cultures. The gonococci were killed by exposure to heat. This vaccine was made up to contain in some cases 50,000,000 and in others 100,000,000 to the cubic centimeter. The material was given by hyperdermatic injection, usually in the wall of the abdomen, occasionally in the back, and usually caused some pain. Few of the children, however, cried on account of the injection, and in the few instances in which some rise of temperature followed it was not regarded as necessarily due to the injection. There were thus given about 220 injections, and a total of about 24,000 million gonococci. No complication or reaction was noted. The children were more than one year old in all but two cases, and none more than 12 years. They all entered the wards with vaginal discharge, the duration of which at entrance could not be determined. They had all passed through the acute stage of scarlet fever, and two of them had complications of a second contagious disease; one had diphtheria and the others measles. It was not thought that the opsonic index could furnish any reliable control of the injections. The dose and times were arranged without reference to the discharge. The treatment and observations were scattered over a number of months, and were made by the writers, assisted by Dr. F. Dietrich and others of the resident staff. During the Spring of 1910 there were chosen from the wards nineteen cases with vaginal discharge, in whom smears made showed gram-negative intracellular diplococci. The diagnosis rested on the examination of the stained smear. Three of these were observed as controls, and the remainder received injections at the rate of three a week. The individual infections varied in size from 25,000,000 to 250,000,000, and the number of injections to the case varied from two to thirty. The duration of observation lasted from one to ten weeks. Notes were made at each injection on the character and amount of discharge present, and it was observed that both in the controls and in those injected the amount of discharge varied extremely from a condition in which the outlet was dry to a condition in which abundant purulent discharge ran freely out and bathed the lips of the vulva. It was observed that this variation sometimes occurred between successive visits, was sometimes explained by the patients getting up from bed, and again was without explanation. No local treatment was given in any case.

It became clear that positive improvement could not be inferred from any single observation, but only from a series of successive observations. Injections were found occasionally to be followed by a notable increase in the amount of discharge, and sometimes by lasting improvement. Two cases, both under treatment over a number of weeks, showed improvement after repeated injections, a relapse when the injections were omitted, and improvement again when the injections were renewed. Other cases presented persistent relapses when the injections had been interrupted.

Observations were also made on fifteen cases treated by a member of the house staff; twelve cases of which were given four small injections of gonococci vaccine and three of which were regarded as controls. Of the three untreated cases all were regarded by the nurses as "improved" during the stay in the hospital, though none of these became quite free of all discharge. Of the twelve examined one to two weeks after the treatment was over, nine showed some moisture at the outlet, two showed thick discharge, and one showed no discharge.

Another series of fifteen cases was studied during the season of 1910. Eleven received injections of fifty to one hundred million at the first injection, and subsequently one hundred million at each injection, and four were observed as controls. Those injected were so treated at the rate of three times per week, and the number of injections per case varied from three to fourteen. Three of the controls showed a practically uniform condition while under observation. The fourth showed considerable improvement. Of the eleven injected cases, seven showed but little change during the period of treatment, and four showed marked variations, and on the whole a little improvement. In no case, however, did the discharge disappear.

In these, as in the previous cases, a smear of the discharge made at the beginning of treatment showed gram-negative intracellular diplococci, and in this series three, four or more smears were made during the period of observation. In all cases but one the smears remained unaltered in character. In one case two positive smears were followed by two negative smears in succession, and those by another positive smear.

Inasmuch as no case was regarded as cured while at the hospital, it was not thought necessary, even if it had been possible, to follow up the cases outside.

NOTES ON THE USE OF POLYVALENT ANTISTREPTOCOCCUS SERUM

MATTHIAS NICOLL, JR., M. D.

Since the first of the year over 30,000 c. c. of antistreptococcus serum have been distributed among physicians and hospitals for use in a variety of septic conditions. During the past five months an attempt has been made to secure clinical data from those receiving it, a written request accompanying each lot of serum for such information in regard to results as would tend to throw light on its value. While such requests have been promptly met in many instances, in an equal or larger number the physicians have failed to furnish the desired information, apparently not appreciating the fact that without coöperation of the clinician little progress can be made in determining the value of this method of treatment. At times physicians from the research laboratory have, when requested, administered the serum and observed the results throughout the course of the illness. There is much evidence at hand to prove that the profession as a whole lack familiarity with the proper use of specific sera, many requests being made for it to be used in totally inappropriate conditions, as, for example, when the culture from the site of lesion or blood shows organisms other than the streptococcus, when the diagnosis is a mere matter of conjecture, or where the patient has apparently but a few hours to live. On the other hand, in not a few instances streptococcus vaccines are demanded for use in cases in which the serum should be employed. Finally the latter is habitually used in too small doses, and if more than one dose is given the subsequent injections are not infrequently improperly spaced. The serum distributed is that which has been in use for several years, prepared from eleven strains of streptococcus taken from the blood, throat and glands of scarlet fever cases and one from a case of malignant endocarditis. During the Spring of 1910 it was extensively used by the writer at the Scarlet Fever Hospital of the Department of Health with fairly satisfactory results. Owing, however, to a limited supply at the time the dosage was on the average not more than half the amount recommended for use by the German and Russian observers who had most experience with it. (See Am. Jour. Children's Diseases, July, 1911.)

Of all conditions for which the serum has been requested puerperal sepsis has been the most frequent. The results obtained by subcutaneous and intravenous injection of varying amounts in this condition have, on the whole, been disappointing. In a number of cases, however, in addition to the general septic condition there have been local collections of pus (abscesses about the uterus or suppurative metritis) which might reasonably account for the negative results obtained. In others no such conditions were present to account for the failure. In a small number of cases not to be distinguished clinically or bacteriologically from the latter the serum seems to have been of decided value, as may be seen from the following examples:

A girl, aged 16, was admitted to the Presbyterian Hospital, having been delivered by forceps six days previously, in a septic condition. Blood culture showed the presence of streptococci. There was a curettage ten days after admission which seemed to have no effect on the general condition or temperature. She received two injections of 50 c. c. on successive days intravenously, together with 200 c. c. of saline. After each injection there was marked improvement in the general condition, the patient making an uneventful recovery, with the exception of a severe general urticarial eruption nine days after receiving the serum, which persisted for four days. (See Chart I.)

E. P., aged 24, admitted to the Presbyterian Hospital April 3, 1911, on the service of Dr. Walter B. James, having had a normal labor nine

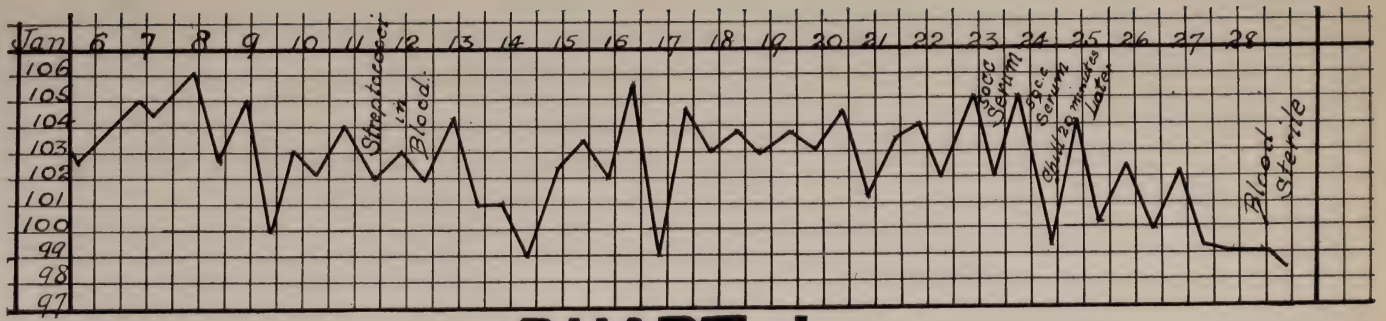

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100
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Streptococci in Blood.
30cc Serum
30cc Serum 30 minutes Later.
Blood Sterile

CHART I

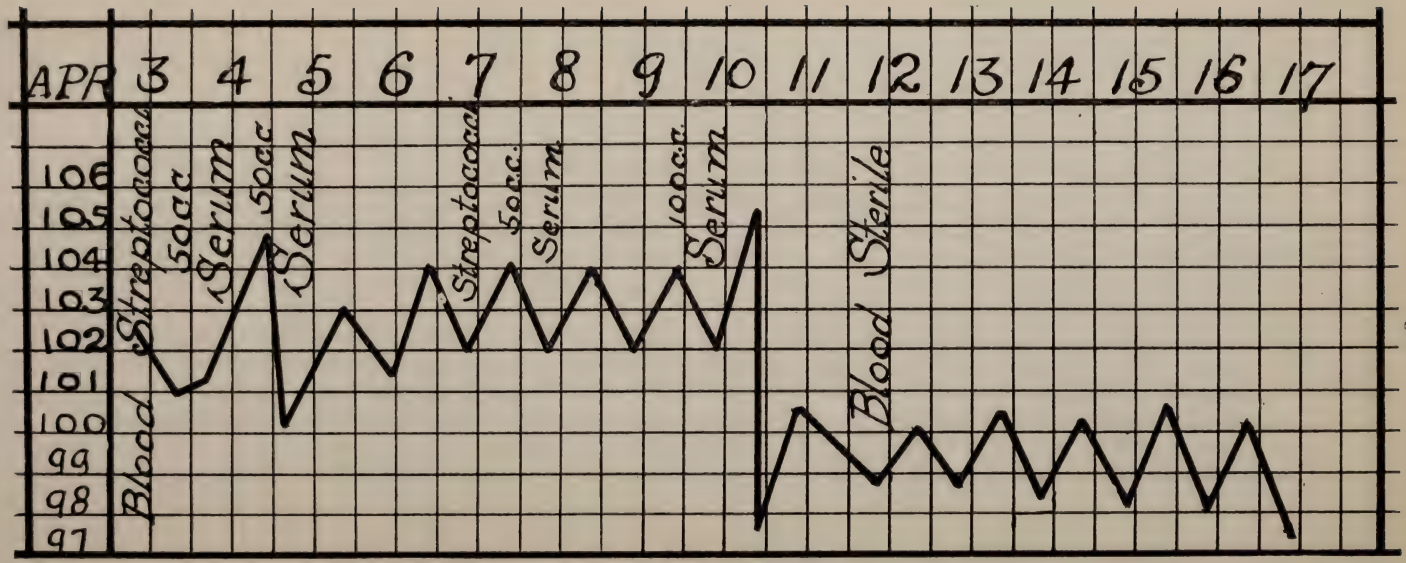

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Soac.
Serum
100 c.c.
Serum
Blood Sterile

CHART II

days previously. On admission she looked acutely ill and prostrated, abdomen distended. The vaginal examination showed a large soft uterus. The blood culture showed streptococci. Four days after admission she developed an acute lobar pneumonia. The first two injections of 50 c. c. of serum intravenously seemed to be without effect. As there was some reason to suppose that the particular serum used had lost more or less of its potency, 100 c. c. of freshly drawn serum was given intravenously five days later. The temperature shortly after fell by crisis. Two days later the blood culture was sterile and remained so, as subsequent examinations proved. The patient went on later to develop an empyema, which was operated on, after which she made an uneventful recovery. Pus from the chest showed streptococci. While the fall of temperature occurring on the fifth day of lobar pneumonia may be looked upon as the crisis of that disease, the fact that the blood became rapidly sterile can be fairly attributed to the use of the serum. (See Chart II.)

The third case occurred in the City Hospital in the service of Dr. Dorman. (See Chart III.) E. M., 14 years, colored, became septic six days after delivery, when considerable placental tissue was removed from the uterus, followed by an intra-uterine douche. Cultures made from the uterine scrapings showed the presence of streptococci. The blood culture was reported negative. Marked improvement followed each injection, with a slight fall of pulse and temperature after the first and more pronounced after the second and third. Urticarial eruption followed the second and third injections, together with some stiffness of the joints. The patient made an uneventful recovery.

The serum has been used in three cases of septic diphtheria in young children at the Willard Parker Hospital, in which after the injection of diphtheria antitoxin there persisted a great deal of swelling of the tonsils and pharynx, sloughing, marked cervical adenitis, profuse nasal and oral discharge and pronounced toxæmia. In all three a very rapid improvement took place in local and general symptoms; nevertheless, two cases died several weeks later, having had a normal temperature and throat for a number of days, evidently of myocarditis, one with a very slow pulse (60), the other with a very rapid one. The third, a very severe case, made an uneventful recovery.

It has also been used extensively for effect upon local conditions due to streptococci alone or associated with other organisms, notably in cases of septic endometritis following childbirth or abortion. The uterus is packed with gauze, saturated with the serum and left for 24 hours, being renewed as often as symptoms demand. In a large number of cases thus treated, notably under the direction of Dr. William E. Studdiford in Bellevue Hospital, there has resulted frequently a rapid fall of temperature and pulse, with prompt subsidence of discharge and restoration to normal condition. Those who have had personal experience are practically unanimous in their belief in the superiority of this over other forms of local treatment.

In other local infections (cellulitis, discharging sinuses, and the like) the good effect of the serum has been very marked in the limited number of cases in which it has been made use of. Its application on saturated gauze causes a rapid subsidence of discharge and stimulates granulations to healthy activity, causing also very prompt relief from the general symptoms due to septic absorption. It may be further noted that there is a decided local anæsthetic property contained in the serum, as testified to by patients who have been thus treated, the local pain diminishing very rapidly after its application. The following may be cited as an example:

A physician's wife, having infected her finger, developed a rapidly spreading cellulitis of the arm and forearm, which extended upward beyond the elbow. This had been thoroughly opened and drained. The granulations two days later looked pale and flabby; pus was pouring from the back of the greatly swollen hand. The general condition of the patient was fair, but her suffering intense. A week after the onset of the

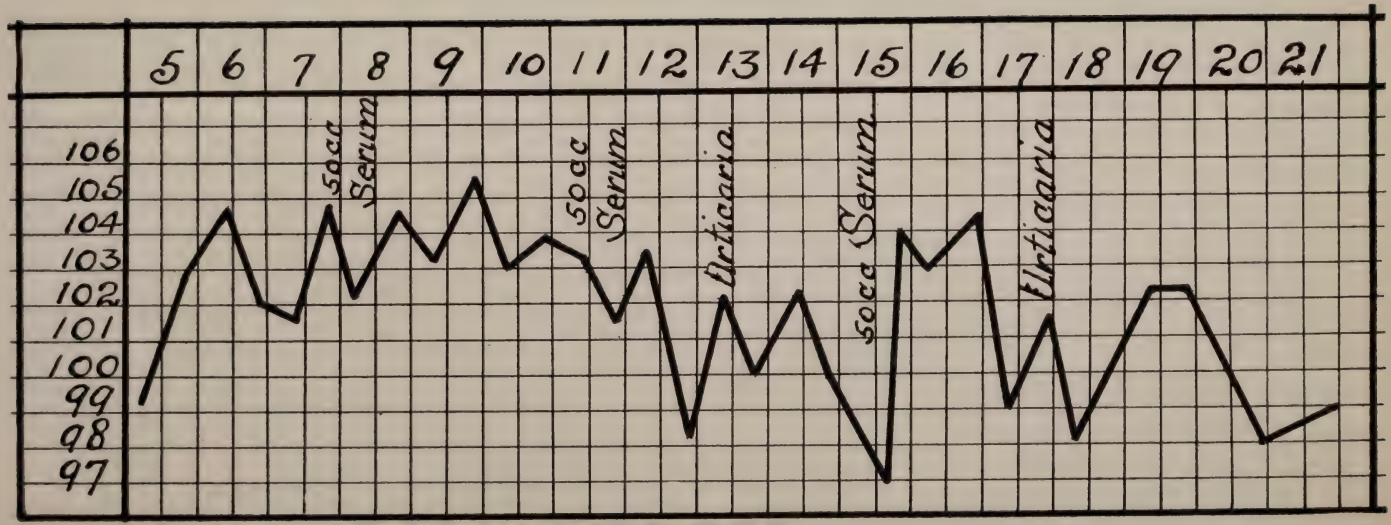

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Soca Serum
Soca Serum
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Soca Serum
Urticaria

CHART III

trouble the sinuses and pockets were flushed with antistreptococcus serum and packed with gauze saturated with it. Two hours later the temperature was 103 at 4 P. M. and at 7 P. M. 99, not rising above 101 during the subsequent course of the illness. The patient experienced great relief from pain shortly after the dressing was made and passed the first good night in a week. On the day following the treatment the swelling had decreased, the amount of pus was markedly diminished and the granulations looked redder and more healthy. Four days later some of the wounds were contracting down, the granulations bleeding and of excellent appearance. The process had involved the extensors of the hand, which will probably result in some loss of function; otherwise the recovery was rapid and complete. Streptococci were found in pus in the wound, but not in the blood.

The serum has also been used locally on the severely inflamed throats of scarlet fever patients by means of sprays or cotton swab. Thus applied at hourly intervals it has produced decided comfort and apparently a much more rapid resolution than would otherwise have taken place.

SERUM COMPLICATIONS:

It would be expected that the injection of such a large amount as 50 to 200 c. c. of heterologous serum, especially when recently drawn, would give rise to symptoms of greater or less severity in many cases, and this has proved to be the fact. Eruptions of an erythematous, urticarial, or mixed type follow the internal use of the serum in many of the cases and when given intravenously appear sometimes with startling rapidity. They may be very extensive and troublesome. Chills, not always but usually due to giving the serum too cold, have been frequently noted. Joint pains with or without a rise of temperature occasionally accompany or succeed the eruption. In one instance a chill, fever, and active delirium lasting for several hours occurred two hours after an intravenous injection of 100 c. c.

Symptoms due to individual susceptibility may be expected to follow the injection of this serum, just as it does occasionally even with the refined diphtheria antitoxin now in use in the Department of Health, usually in persons of an asthmatic habit, with the immediate production of spasm of the bronchi, arterioles, œdema of the glottis and lungs. These symptoms are always alarming and occasionally fatal, and if there is anything in the previous history of the patient which would lead the physician to anticipate such a result, serum injections had better be avoided except in cases of grave necessity.

True, general anaphylaxis is unlikely to follow the usual methods of administration. The writer has reported one instance of it, not fatal, in which the patient became comatose after receiving a few drops of antistreptococcus serum intravenously, diphtheria antitoxin having been given three months previously. (See reference above.) A probable instance of local anaphylaxis of a severe type occurred in a patient who under ordinary circumstances would have been considered immune to serum. A woman was admitted to the service of Dr. R. H. Wylie in Bellevue Hospital on April 7, 1911, suffering from puerperal septicæmia following instrumental birth of a child three months previously. The blood showed streptococci with a high polymorphonuclear leucocytosis. She was given serum by deep intramuscular injection in amounts and intervals as follows: (See Chart IV.)

The last injection given deep into the thigh was followed in a few hours by the formation of a broad red areola about the site. A chill preceded this phenomenon and was repeated on the following day. The area became rapidly swollen and indurated, the patient being kept under the influence of morphine on account of the pain. In two days the tissue broke down, leaving a slough which involved the tissues down to the underlying muscle, and being six to eight inches in diameter. Granulation took place slowly, the wound not having completely healed on the patient's discharge from the hospital six weeks later. No further injections were

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
C.	C.	50	50	50	50	30	60	75			75			50							50	50	60	70	80	90	100	110	Reaction	Slough

CHART IV

given, as the blood proved to be sterile two days after the final one; the patient recovered and left in good condition. The explanation of this phenomenon will have to be determined by future experience, such a long series of injections being quite unique. It is probably a clinical example of what is known experimentally as Arthus phenomenon (a reaction of certain tissues to a foreign serum, due to an unexplained lack of local immunity). It should be said, however, that the patient was extremely emaciated and there is the possibility of the tension produced by the serum, together with a moderate reaction in poorly nourished tissues, being the cause of the slough. On several occasions previously an urticaria had followed the injections; otherwise, there had been no reaction other than that recorded.

DOSAGE AND METHODS OF ADMINISTRATION:

In the type of disease in which the serum is indicated a more or less rapid result is essential if life is to be saved, and for this reason an intravenous injection would seem on theoretical grounds at least to be the ideal method of administration. This, however, has not been used in a sufficient number of cases to enable us to draw conclusions as to its superiority over the subcutaneous method, which has been practiced notably in Russia and Germany on a very large scale. It may be stated, however, as the result of a limited experience that the injection of large amounts of serum directly into the vein may be rapidly followed by very disagreeable and sometimes alarming results. In peculiarly sensitive individuals the rapid introduction under such conditions will be more likely to be followed by serious results than when the serum is given subcutaneously. It is therefore advisable, except when the element of time is of the utmost importance, in which case the quicker method should unhesitatingly be adopted, to give the serum by the latter method; or, again, when an apparatus suitable for giving the latter is not at hand, *i. e.*, one suitable for forcing a large amount of serum into the tissues. In giving an intravenous injection it is not often necessary to cut down on the vein except in the case of very young children. It should be warmed to 100° F., and given pure or diluted with an equal amount of normal saline. There is little danger of its running too freely unless an unusually large needle is used. The apparatus used by Dr. Park for the injection of large amounts of antitoxin in horses meets all the requirements of this operation, as well as those of the subcutaneous method. It consists of a glass tubular graduated container holding 200 or 300 c. c., with a tight fitting rubber cork, pierced by glass tubing, to which is attached a hand-ball pressure bulb; three or four feet of rubber tubing are attached to its lower end, a suitable needle being fitted to the end of the tube by a glass attachment. The latter should be inserted separately in the vein and blood allowed to fill its caliber. The serum is then permitted to fill the tube throughout its whole length, and the latter attached to the needle, the serum then flowing by gravity into the vein at a rate which may be regulated by raising and lowering the apparatus. For subcutaneous use the needle is inserted deep in the tissues of one side of the lower abdomen and the serum forced in slowly by means of the bulb. It is desirable to inject not more than one hundred c. c. in one place. If a larger dose than this be given the opposite side of the abdomen may be used. The swelling caused by this amount of serum is formidable and the tension at times is very great, but after the first 10 or 20 c. c. are injected there is no pain and very little discomfort, the serum being rapidly absorbed. In general the dosage by either method should be for adults 150 to 200 c. c., to be repeated by a dose of one-half the amount in from 24 to 48 hours. Whether further injections are indicated must depend on the character of the case, the result obtained, and the amount of reaction, if any. For young children the dose should be about one-half of that given for adults.

In conclusion it would seem probable that more favorable results than those described may be looked for from the use of a serum obtained

by the inoculation into horses of strains of streptococci taken from every available septic condition due to this organism, especially as there is reason to suppose that certain pathogenic differences may exist in this group. Such a serum is now in the course of preparation in the Department of Health.

The writer desires to express his thanks to the physicians on whose service the cases cited occurred for permission to quote the records, and to Drs. E. R. Evans, of the Presbyterian Hospital, and R. H. Thomas, of the City Hospital, for their assistance in preparing them.

NOTES ON NOMA, WITH A REPORT OF A CASE TREATED WITH SALVARSAN

Matthias Nicoll, Jr., M. D.

During the spring and early summer of the present year there occurred an unusually large number of fatal cases of noma in the City of New York.

At the Scarlet Fever Hospital of the Department of Health there were 11 cases and 9 deaths. So much confusion exists in the nomenclature of the various ulcerative conditions about the mouth, especially in children, that it would seem expedient to arrive at some understanding as to what pathologic condition may properly be termed noma. Thus, small ulcerations occur on the inner surface of the cheek from which smears show the presence, often in great numbers, of the characteristic fusiform bacilli and spirochætæ, but which, nevertheless, readily yield to the application of caustics or milder local remedies. Other cases, not to be differentiated bacteriologically from the latter, do not yield to treatment, the ulcero-membranous process extending with startling rapidity to the gums and then to the neighboring alveolar process of the jaw. Again, marginal ulcerations about the teeth, together with a spongy condition of the gums, yield similar bacteriologic findings, but in otherwise healthy children do well under treatment by local application.

The so-called Vincent's angina, in spite of very widely extended lesions, usually produces but few general symptoms, and, after a somewhat tedious local treatment, runs a favorable course without extensive loss of tissue.

For the purpose of these observations, the term noma is to be understood as designating a rapidly-spreading gangrenous process, beginning usually on the mucous membrane of the cheek and gums and involving all surrounding tissues, including bone, showing little tendency to self-limitation, and from which fusiform bacilli and spirochætæ may be obtained in great numbers in the smears.

The cases at the Scarlet Fever Hospital involved only the mouth and face, although the same process may occur about the external auditory meatus, rectum and vulva.

Observations of this disease, extending over many years, especially at the New York Foundling Hospital, together with the study of most of the 11 cases referred to above, justifies the writer in arriving at the following general conclusions:

(1) The disease is essentially one of contagious disease hospitals and institutions for young children, but few typical cases having been reported from other sources.

(2) It is very largely confined to children under five years of age.

(3) It attacks almost wholly those suffering from marked malnutrition, and whose resistance has been lowered as a result of previous disease.

(4) Measles has long been regarded as one of the predisposing causes. In the 11 cases mentioned, 4 had an antecedent attack of measles during their convalescence from scarlet fever.

(5) During the occurrence of an epidemic of noma a large number of minor ulcerative or ulcero-membranous conditions are found about the mouth and gums in otherwise healthy children, showing the same organisms as those found in true noma, but showing little tendency to spread.

(6) In the epidemic at the Scarlet Fever Hospital there was no positive evidence of direct contagion, the cases being scattered over five floors of the building.

(7) Occurring as it does in patients in a low state of nutrition, the disease shows little tendency either to spontaneous recovery or to yield to local treatment, or even be influenced by radical surgical measures —curetting and removal of diseased tissues. Occasionally a sequestrum of dead bone is formed, sharply marked off by healthy tissues and spontaneous healing occurs. In the 11 cases mentioned one took this course—that which occurred in a patient in previously fair general health. In another the same process was seen after treatment by salvarsan. The other 9 died after treatment, which varied from the application of strong caustics and frequent cleansing of the parts to removal, under an anæsthetic, of all the visible diseased tissue, including bone. It cannot be said that radical measures had any apparent effect on the progress of the disease. Indeed, in some cases they seemed to act adversely.

The case treated by salvarsan is as follows: Robert M., aged five, admitted to the Scarlet Fever Hospital June 10, 1911, on the fourth day of his illness—a well-developed and well-nourished boy, with the typical symptoms of scarlet fever of a severe type. The heart was normal; the

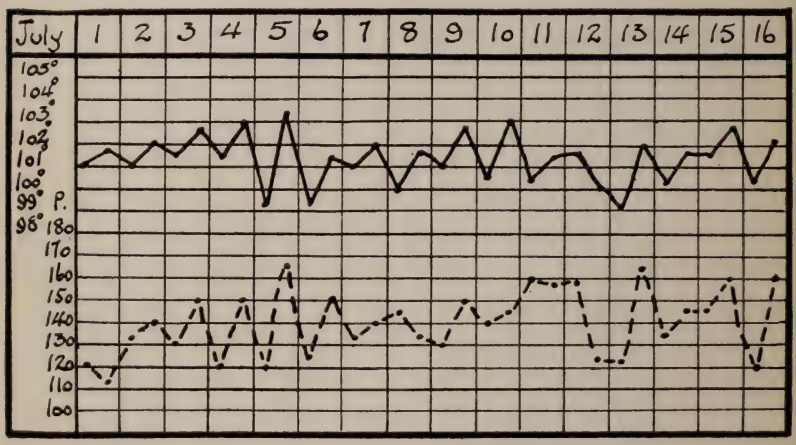


Chart Showing Pulse and Temperature.

lungs showed the presence of a moderate bronchitis. He was said to have had measles some time ago; otherwise his health had been good. There was a pseudomembrane on the right tonsil, from which cultures did not show the presence of Klebs-Loeffler bacilli. On admission he was given 5,000 units of antitoxin, and on the following day 10,000 units. There was a profuse discharge from the nose and mouth. For forty-eight hours after admission he was delirious a good deal of the time, with a rapid pulse and high temperature. June 15th: The condition was very much better; temperature and pulse had fallen, the discharge very much decreased. June 23d: The cervical nodes had been swollen from the first, gradually increased in size and were now very tender. June 24th: Glands incised and drained. The temperature had been keeping up, evidently due to the cervical adenitis, but fell twenty-four hours after the operation. June 29th: Both ears discharging. July 4th: There was an ulcer on the inner side of the right cheek, one-eighth inch in diameter, opposite the upper molar teeth, and another similar one opposite the lower molars. The gums were not involved. July 5th: Gums bleeding and spongy, the ulcers now having extended to them. There was another ulceration on the left cheek opposite the upper molars. The right cheek

was very much swollen, the eye closed, the left cheek less so. Both swellings were of stony hardness. The patient's general condition had become very bad. He was greatly emaciated and very anemic. The odor from the mouth was very offensive. In the afternoon of this day he was given three-tenths of a gram of salvarsan in 200 c. c. of diluent intravenously. July 6th: The swelling of the cheeks on both sides had increased very markedly, the skin being tense, shiny, and somewhat red. The bones of the jaw were exposed both above and below on the right side, denuded of periosteum and black. Between the bare bone and neighboring gum there were ragged, sloughing walled sinuses running up and down for some distance. Pieces of broken-down tissue came away with irrigation. Smears from the original ulcers showed the fusiform gram-negative bacilli and spirochætæ; later smears, mixed bacteria. Local treatment consisted in frequent cleansing of the pockets, packing with gauze saturated with various antiseptics and with antistreptococcus serum. July 9th: The condition was apparently stationary. The sinus in the left upper jaw runs up to a point opposite the parotid gland. Large amounts of broken-down tissue came away through this, some apparently of the gland substance. July 11th: The local and general condition continue the same, and, while it was apparent that the disease was making no characteristic advance, it was thought advisable to repeat the injection of salvarsan in the same dosage. July 12th: The same local reaction about the swollen right cheek was noted twenty-four hours after the injection. The swelling of the right cheek had been steadily diminishing and was now but little marked. July 16th: There was a fine hæmorrhagic eruption on the face, hands and legs resembling purpura. July 20th: The eruption was still present, but fading. The teeth opposite the lesions are all loose. The sequestra of bone are being sharply delimited from the healthy. August 15th: The boy was out of bed part of the day; had been gaining rapidly in general health and strength. The lesions were cleansed regularly. September 18th: The patient's general health is excellent. It was not thought advisable to remove the loosened sequestra, which involved the alveolar process through one-half of its diameter, approximately opposite three teeth of the upper and lower jaws on the right side, and upper on the left. Running up from the latter the sinus is still open for a short distance, but is clean. A slough in the bend of the left elbow caused by the leakage of some of the salvarsan into the tissues has completely healed without loss of motion. October 10th: The sinus has been healed for several weeks; the boy is round about the hospital in excellent condition.

The effect on pulse and temperature following the injection of salvarsan is shown in the accompanying chart. The pulse remained fairly rapid for several weeks thereafter; the temperature slightly elevated, 99° to 100½° F. The noticeable local reaction, consisting in increased swelling and redness in the infected cheek twelve hours after each injection, was also observed in another case following each of two injections and which did not recover. The latter was one in which the disease made an unusually rapid progress and involved both sides of the face in a patient suffering also from severe scarlatinal nephritis, with marked œdema of the face and extremities.

The writer has not found any recorded instances of salvarsan having been used in a true case of noma. In allied conditions it has been given apparently with beneficial results. Thus Rumpel employed it in doses of 3 to 5-10 grams in 7 cases of Vincent's angina, 3 of which were negative to Wassermann, the others not having been tested. Healing took place in five to sixteen days without other treatment, the organism disappearing from the smears within from four to twenty-five days. Gerber also made use of it successfully in various forms of ulcerations about the mouth, including scurvy, which showed the typical organisms.

Had it been proven that the spirochætæ found so abundantly were the specific cause of the disease, there would be no good excuse for not giving salvarsan as a matter of routine in all cases whose physical condition would lead one to fear the occurrence of noma, and, while the evi-

dence of such a causal relationship is yet but circumstantial, it is very convincing.

Organisms not to be distinguished morphologically may be found not infrequently in apparently normal mouths, but rarely in large numbers. Such was the case during the epidemic of noma at the Scarlet Fever Hospital. Dr. McDonald, the assistant resident physician, examining a large number of cases, found the fusiform bacillus and spirochætæ often in preponderating numbers in smears from various types of stomatitis, especially in those of an ulcerative character.

Lately Blühdorn has found the two organisms, sometimes the bacilli, sometimes the spirochætæ, predominating in diphtheria and scarlet fever throats, syphilis, ulcerative stomatitis, Vincent's angina, and normal throats. He believes that these organisms normally inhabit the mouth in health, but increase greatly under certain pathologic conditions.

Gerber thinks that stomatitis of a membranous and ulcerative nature is probably due to a symbiotic growth of the organisms, and finds that the spirochætæ may readily be recognized in the dark field.

Lately Ruth Tunnicliff has made a careful study of these organisms, and reaches the following conclusions: "Strains of fusiform bacilli isolated in pure culture from the normal mouth, ulceromembranous angina, gingivitis and noma appear culturally and morphologically to be the same organism. The cultures show that the bacilli and the spirochætæ are different forms of the same organisms. Whether the spirilla formed from the fusiform bacilli are the same as those found in the lesions themselves cannot be decided on account of the inability to reproduce the lesions in lower animals."

She found that the use of killed cultures of fusiform bacilli and spirochætæ in a case of noma caused an increase in the amount of specific opsonins and appeared to produce some beneficial effect.

In conclusion it would seem advisable even in the absence of positive evidence that spirochætæ are the specific organisms of noma to make use of salvarsan in every case which does not immediately yield to local treatment, for it cannot be denied that other remedies have had little or no effect in controlling this horrible disease and the use of salvarsan is at least logical.

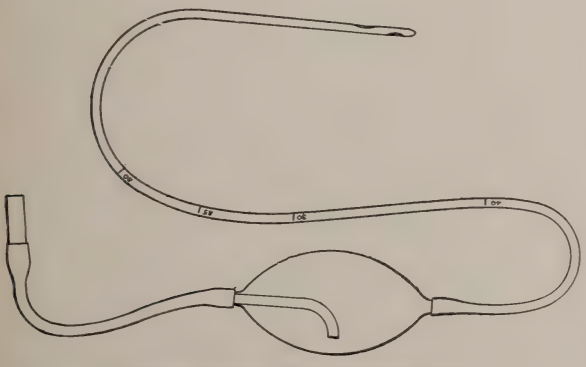
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THE USE OF A SIMPLE DUODENAL CATHETER IN THE DIAGNOSIS AND TREATMENT OF CERTAIN CASES OF VOMITING IN INFANTS

Alfred F. Hess, M. D.

Some months ago I described in the pages of this journal[1] a duodenal tube for infants. This tube was introduced by mouth into the stomach, and then passed through the pylorus into the small intestine. This result was readily accomplished, the tube taking about one-half hour to pass the pylorus. The radiographs which accompanied this article showed conclusively that the end of the tube lay in the duodenum, and was not merely curled on itself within the stomach cavity. This duodenal tube, in brief, consisted of soft rubber tubing with a silver-plated, perforated leaden ball at the end, and was a modification of the Gross duodenal tube used for adults. By means of this simple device I was able, after some practice, to gain access to the duodenum in infants in almost every instance, and gradually acquired increased facility in its use. This was, I believe, the first conclusive proof that the intestine could be reached by this route in infancy. The principle involved in this manipulation was that of gravity, carrying the leaden ball to the pylorus, aided by the onward movement of peristalsis, which swept the ball through the pyloric ring, and thence into the intestine. Although this tube served its purpose, I have laid it aside for one which is simpler, as well as more serviceable,



Duodenal Catheter With Aspirating Bulb.

and which has afforded me a ready means of studying the functions of the stomach and intestines from a new point of view.

In the course of my experiments it occurred to me to try whether I could obtain the same result by making use of the ordinary soft rubber Nélaton catheter, which is used so frequently in infants and in adults for the purposes of lavage and feeding. To my gratification and surprise I found that this simple instrument can be passed readily from the stomach into the duodenum. It is indeed interesting and instructive to reflect on the fact that this catheter, which was introduced into the realm of pediatrics by Epstein in 1880 for the purpose of gastric feeding and investigation, has, in these many years, never been seriously thought of in connection with the diagnosis and treatment of pyloric conditions and of

[1] Hess, A. F.: A Duodenal Tube for Infants, Am. Jour. Dis. Child., May, 1911, p. 360.

intestinal disturbances, subjects which have been considered of primary importance during this long period.

During the past few months I have made use of this catheter, which may in this connection be termed an "infant duodenal catheter," for these purposes on more than 200 infants of various ages, suffering from a great variety of disorders, and can at this time speak with confidence of its practicability in this connection.

In order to demonstrate beyond all doubt that the catheter gains access to the duodenum, I have once more resorted to radiographic pictures (Fig. 1), criteria which are least open to doubt, and which



FIG. 1.—View from posterior. Duodenal feeding of 3-months-old infant; stomach empty; bismuth-milk mixture in coils of intestine.

have enabled me to prove this point conclusively. From this picture, for which I take pleasure in thanking Dr. Hirsch, it will be seen that the tube is well within the intestine; in fact, in one instance, seems to have passed even beyond the confines of the duodenum. It will be noted also that the catheter does not take a direct course from the cardiac end of the stomach to the pylorus, but that just as in the case of the duodenal tube, which I previously described, the catheter on entering the stomach cavity makes a sharp bend to the left to the fundus, and then turns on itself to the right to reach the pyloric opening. This has been the course followed in all radiographs which I have had taken, either of this catheter or of its predecessor, the duodenal tube. This novel and unexpected picture controverts the assumption which I first considered, to explain the ease with which I was able in infants to reach the pylorus. It shows that this success cannot be attributed to the vertical position of the stomach in infancy, nor to the fact that the tube or catheter follows an almost vertical course in its route to the small intestine, and has led me to attribute the success of the procedure rather to the diminutive size of the stomach cavity, and also perhaps to a relative lack of tonicity of the pyloric ring. This explanation gains added weight from the fact that a catheter of this kind cannot be used for this purpose in the adult, in whom the stomach is large, and the tube wanders far from the pyloric opening.

TECHNIC

It is difficult to describe the method of use of this simple instrument, for like all other instruments, even of a simple nature, its use can be learned only with some practice, and the descriptions are far more complicated than the procedure would warrant. For the purpose an ordinary No. 15 (F.) soft rubber catheter should be employed, preferably one with a large eye; if the baby is very small, under a month of age, a No. 14 (F.) catheter should be made use of. Before beginning we should designate points 20, 25 and 30 cm. distant from the eye. This can be done by means of pen and ink. These markings allow us to take our bearings while introducing the catheter, and as long as it is in place. The catheter is now ready for use. The only other instrument necessary is an aspirator; for this purpose either the same aspirating bulb which I described in my previous article may be used, or an ordinary aspirating syringe. The instrument is now complete. The physician, therefore, generally has an infant duodenal catheter at hand, and needs but to acquaint himself with its use.[1]

The catheter is passed in the same way as the ordinary stomach tube, that is to say, it is lubricated with water, introduced into the mouth, and without the aid of the finger is rapidly pushed down the pharynx and œsophagus into the stomach. It has seemed immaterial to me whether the baby is held in the sitting position, or lies on its back, or on the side. I have generally had it placed on its back with the arms bound snugly to its side by means of a towel. In a baby 2 months old, for example, when the 20 cm. mark is about 3 or 4 cm. from the gums, the contents of the stomach begin to be evacuated. This food is generally acid, and may contain free hydrochloric acid, as shown by its reaction to congo paper, if the feeding has been given some hours previously. After the stomach is empty, if we insert the catheter gently a little further, we shall find no resistance until the 20 cm. mark is about at the gums, when

[1] Although a serviceable instrument can be prepared in this way by anybody wishing to make use of one, and a great deal of my work was carried out by means of a catheter which I happened to have on hand, nevertheless a catheter with two eyes at the tip, of exceptional length, and carefully marked, has additional advantages. The use of the bulb instead of an ordinary aspirating syringe has decided advantages; the greatest is that in aspirating through the bulb by means of the mouth one can in this way appreciate the amount of resistance offered by the stomach, or intestine, and thus gain valuable information as to the whereabouts of the end of the catheter. I should by all means urge anyone who intends to use this method frequently to make use of an aspirating bulb. The bulbs and the infant duodenal catheters may be obtained of Tiemann & Co., New York.

a sense of obstruction may be felt, and the infant may gag, due to the contact of the end of the tube with the pylorus. While there is gagging or crying, accompanied by forced expiration, the tube should never be advanced, for at this time the pyloric valve, and indeed the cardia as well, is constricted and prevents further passage. If we push on a little until we reach the 25 cm. mark, we shall in most cases by this simple means have gained access to the duodenum. Indeed I have no doubt, and a recent perusal of the literature has confirmed me in this opinion, that many have reached the intestine when they wished merely to enter the stomach, and I believe that numerous reports which are supposed to record the capacity of the stomach, or to describe the nature of its contents, in reality combine data of the contents of this viscus and that of the upper part of the small intestine as well. When we consider also that pressure of the tip of the catheter on the pylorus at times causes the pyloric valve to allow duodenal contents to pass into the stomach, there is further reason for believing that errors have crept in.

Naturally, the first question is "How do we know that we have reached the intestine, and why may not the catheter be curled on itself within the stomach?" Indeed it is far easier to enter the duodenum than to learn the phenomena by which we may judge whether or not we are in the intestine; however, the more experience we gain the easier it becomes to judge of this point, and for some time I have felt very little uncertainty in this regard. There are several ways of ascertaining this fact. In the first place we must have an approximate knowledge of the distance of the pylorus from the gums or teeth. For example, this measurement for clinical purposes is about 20 cm. at one month, gradually increasing to about 25 cm. at a year.

Let us suppose that we are passing the duodenal catheter on a baby 3 months of age, and that we have gradually inserted it to the 25 or 30 cm. mark. We then halt and watch to see whether the tube has a tendency to remain at this level or not. If you push a tube into the stomach, and have it coil up within its cavity, it will almost always, sometimes quickly, sometimes more slowly, the time depending on the fact of whether the child is quiet, or gags and resists, work its way out of the mouth, so that the 20 cm. mark comes into view again. If, however, the tube has passed into the duodenum and is held by the pyloric sphincter, it will project almost vertically from the mouth and have a tendency, unless there is marked gagging, to hold its position, and not to work its way out again. This is an important, although not pathognomonic, sign of having reached the intestine. Frequently we are aided in obtaining our bearings by feeling the grip of the pylorus, an intermittent closure, when we pull slightly on the catheter.

It might be thought that one of the most useful tests for orientation would consist in obtaining alkaline fluid or bile on aspiration, and most certainly if we obtain such fluids the evidence of success is convincing, and the gratification proportionately great. However, unless we are prepared to spend one-half hour, or an hour, or at times a still longer period, we may be unable to demonstrate our success in this manner. Nor is this necessary. The duodenal contents are frequently acid, and may continue to be so, due to the fact that gastric juice is being poured out into the stomach and thence into the intestine. This may render it impossible to obtain alkaline juice, although the fluid may be golden yellow, due to bile, and contain the various pancreatic ferments. Moreover, we may obtain a neutral or alkaline juice without any bile, as the bile is excreted intermittently, and often at long intervals, so that if we were to depend entirely on the reaction of the fluid or on its bile content, in judging of our success in reaching the intestine, we would often consider ourselves unsuccessful, whereas in reality we had reached the desired goal.

A test which is of value, especially in the beginning of the use of the catheter, is what I have called for the sake of brevity the "retraction test." By this I mean that when we are in doubt as to whether the catheter is in the stomach or duodenum we retract it very slowly, having

an assistant aspirate through the bulb as we do so, until we have re-entered the stomach cavity. In this way, if the catheter has been withdrawn from the duodenum, one of two signs may be noted: either the assistant may note a marked change of resistance to aspiration, as the end of the catheter leaves the small constricted intestinal cavity and enters the stomach (and indeed frequently the assistant may give the first signal that the tube has left the intestine), or it may be noted by the sudden change in the nature of the juices obtained. Whereas, when the tube was in the duodenum a small amount of viscid fluid, neutral or somewhat acid, was being aspirated, or, indeed, no fluid could be obtained, when the tube is withdrawn to, let us say, the 20 cm. mark, suddenly there is a noisy gush of fluid of an entirely different nature, perhaps containing a marked admixture of foamy saliva, generally much larger in quantity, and frequently far more acid than that which had been obtained in the duodenum. As I have said, this test tells us whether the catheter was in the duodenum or not; however, it necessitates our making a second attempt to pass the pylorus. The more we use the tube the less we have to resort to this test.

Another test, but one of much less value, is what I have termed the "papillary bile-test." By this I mean that sometimes when we withdraw the tube from the intestine toward the stomach, and reach the level of the papillæ of Vater, there is a sudden gush of bile-stained fluid. This, however, is not usually the case, and the test cannot be relied on, but should be remembered, and made use of, in case this sign is noted in the course of the "retraction test."

Like most manipulations, the passage of the duodenal catheter requires some practice and cannot be learned by description, but only by personal experience. I have found that after a few attempts the technic was fairly well acquired by hospital interns. The simplest method of acquiring the technic is to pass the catheter in the way described, and control the result by means of the fluoroscope (the catheter casts a deep shadow without the aid of bismuth). However, the *x*-ray is by no means necessary, and I did not make use of it in gaining my experience. Practice—far less practice than is required to master the technic of an otological examination—is all that is required. Indeed, in very young infants it is difficult not to enter the duodenum if we do not push the catheter forward with too great haste. The younger the infant the easier is this accomplished. I have passed the catheter without difficulty on infants ranging in age from a few hours to one and one-half years. However, in a broad sense it may be stated that the younger the infant the easier it is to transgress the pyloric barrier.

In this paper, in addition to describing a simple method of reaching the intestine in infants, a technic which seems to me to offer additional possibilities for diagnosis, for therapeutics and for physiologic experiment, I wish to describe how this procedure has been of value in the diagnosis and treatment of some pathologic conditions which I have encountered during the past few months.

THE CATHETER IN DIAGNOSIS

Let us consider the subject of diagnosis. There is perhaps no group of cases more puzzling at times than that characterized by persistent vomiting. From day to day our diagnosis wavers among various possibilities—chronic indigestion, pyloric spasm, beginning pyloric stenosis and other less common and even less well-defined conditions thrust themselves forward in turn for consideration. For, although the diagnosis in the classical case is simple, in many others we grope for weeks, and even when all is over we do not feel confident that we have solved the nature of the disturbance. In cases of this description the duodenal catheter, and at times the soft rubber ball-tube as well, has been of assistance. To cite an instance:

Case 1.—A. W., aged 2 months, weight 5¾ pounds, a premature baby, with a history of vomiting for some weeks, was admitted to Dr.

Caillé's service at the Post Graduate Hospital, May 10, 1911. After admission the infant regurgitated almost all feedings; temperature was subnormal, stools yellow. Food consisted of a milk-mixture containing approximately 1 per cent. fat, 6 per cent. sugar, 1 per cent. protein, of which 3 ounces were given every three hours.

May 13, 4 p. m. Catheter passed and stomach emptied of about ½ ounce of watery fluid. Some difficulty in passing tube into stomach. Duodenum could not be entered. The duodenal ball-tube (6 mm. ball) was also tried without success. Both were held at the 20 cm. mark.

At 4:25 p. m. an attempt was made to allow the tube to find its way through the pylorus by the slow method, by peristalsis. The cardia, and more especially the pylorus, seemed irritable, the baby crying when the tube was pushed beyond 20 cm. mark. Five c. c. of markedly acid juice was obtained. At 5:30 water was put into the stomach to aid instrumentation, but of no avail; held always at 20 cm. mark. At 5:35 the tube was removed.

Epicrisis: A case of spasm or stenosis of the pylorus, combined with spasm of the cardia. Nothing was known of the history of vomiting of this case when the catheter was passed, as the infant was selected for the purpose of obtaining duodenal juice for chemical examination. It was only after our unexpected failure to pass the pylorus that we inquired the anamnesis, and learned to our great interest of the persistent vomiting. We then put 4 ounces of fluid into the stomach by lavage, but no marked gastric peristalsis was noted; 1 ounce of this fluid was forcibly ejected.

May 16, Test 2: Baby still regurgitates; food the same; fed two hours ago; temperature subnormal; stool yellow.

3:30 p. m., 6 mm. ball-tube passed; obstruction at 15 cm., which was overcome; marked acidity of food in stomach; ball again held at about 21 cm. mark, even though water was put into stomach (accessory method).

4:05, tube withdrawn, some obstruction at cardia.

4:10, 16 (F.) catheter passed, slight obstruction at 15 cm.; marked obstruction at pylorus.

4:35, marked gastric secretion; ball-tube again tried, after dipping it into cod-liver oil and pouring a small amount of oil into stomach. Failure.

Epicrisis: Again failure to enter duodenum, obstruction at cardia and at pylorus as previously noted.

May 18, Test 3: Child has vomited less since last passage of tube. Is getting mixture of milk containing approximately 2 per cent. fat, 6 per cent. sugar, and 1 per cent. proteids.

4:15, No. 12 (F.) catheter introduced into stomach, and a small quantity of very acid Congo + fluid evacuated. Obstruction at cardia at 15 cm. noted as previously.

4:45, tube reintroduced, and at once golden, faintly alkaline fluid was obtained; 8 c. c. aspirated with the tube introduced 22 to 27 cm. (This fluid was found to contain the pancreatic ferments, amylase, protease, and lipase in considerable amounts.)

5:10, tube removed. Pylorus probably at 21 cm. level.

5:30, 6 mm. ball-tube introduced. This could not be advanced beyond the cardia without having the baby swallow water. In 10 minutes it did not pass the pylorus.

Epicrisis: At the third test we were able to enter the duodenum, as proved by aspirating bile and a large quantity of fluid containing all the pancreatic ferments. The pathologic condition in this case, therefore, would seem to have been a spasm of the pylorus and cardia, rather than an organic stenosis. If a stenosis did exist, it could not have been present to a marked degree.

May 27, Test 4: Baby still vomiting repeatedly. Six mm. ball-tube passed through the cardia and the pylorus by means of having the baby swallow water (accessory method). Bile obtained, and a large amount (6 c. c.) of pancreatic juice containing the three ferments. In duodenum thirty minutes.

June 1, Test 5: Baby still has propulsive vomiting. Six mm. ball-tube passed with difficulty (accessory method); a marked grip of the pylorus noted; bile obtained; tube then withdrawn.

June 9, Test 6: Infant had been given paregoric to stop the vomiting, but without effect. Condition very poor. Weight, 4 pounds 12 ounces. 4:30, 6 mm. tube passed through pylorus by the aid of water, as proved by the "retraction test."

4:55, reintroduced. Two ounces of milk-mixture fed slowly into duodenum.

June 10, Test 7: 10 a. m., inserted tube into duodenum again; 11 a. m., 8 c. c. of bile-colored fluid obtained in one hour. This fluid was alkaline, and contained the three pancreatic ferments. Pyloric tugging marked when tube was withdrawn from duodenum.

In all, seven tests were carried out on this infant. In the first two attempts we were unable to pass the catheter into the intestine. In the others this was accomplished with difficulty, and only by the aid of introducing water into the stomach. This technical resort is often very helpful, as the pylorus relaxes to allow the water to pass through, and in this way the catheter also obtains access to the duodenum. For the sake of brevity, this device, for which we do not claim originality, has been referred to in the notes as the "accessory method." In this case where a spasm of the cardia as well as of the pylorus existed, this method proved especially helpful in relaxing both sphincters. It is of interest to note that in addition to the increased amount of gastric secretion which has so frequently been observed in conjunction with pyloric spasm, there was in this case, as evidenced in the second and third test, an increased flow of pancreatic secretion as compared with the amount usually obtained. At the time this case presented itself for examination we had just begun to use the duodenal catheter, and were still employing the ball-tube. It is very probable that had we used the catheter in the examinations we should have been able to enter the duodenum more readily.

We were not in a position to attempt systematic duodenal feeding of this infant; however, the one attempt made at the time of the sixth test showed that this was feasible, and it is to be regretted that the baby could not have been given alimentation continuously by this route.

It is not our purpose to give in detail an account of all the cases of pylorospasm which we have tested by means of the duodenal catheter, nor, indeed, to discuss in this place the nature and peculiarities of this interesting condition. We have passed the catheter on numerous infants, who on account of persistent vomiting were suspected to be suffering from this condition. In many of these cases the catheter was readily passed to and fro between the intestine and the stomach, so that the diagnosis of spasm could readily be set aside. On others a definite obstruction was encountered. One of these has specific points of interest, and, therefore, may well be recounted in detail:

Case 2.—M. S., 3 months of age, with marasmus; never nursed; had vomited a great deal between June 27 and July 20. Gavage and paregoric used. Not vomiting at present. Weight, 4 pounds 5 ounces. Fed every two hours 3 ounces of a mixture containing one-half boiled milk, one-half water, and 5 per cent. sugar; had been fed two hours previous to test, which was carried out to obtain pancreatic ferments.

August 18, Test 1: 2:20, catheter introduced into stomach, which was found to be empty. 2:25, No. 14 (F.) catheter passed readily into duodenum. Some warm water was given. In the course of thirty minutes about 13 c. c. of alkaline, greenish, slightly viscid fluid was obtained (subsequent examination showed this to contain lipase and protease to a marked degree; the amylase test was lost). 12:55, tube removed. Clear acid fluid obtained in stomach.

Epicrisis: A normal case except for the excessive secretion of duodenal fluid.

September 12, Test 2: Weight, 4 pounds 8 ounces. Appears nervous and cries a great deal. Since September 3 infant has been vomiting, and

for the past three days has vomited all food. It is given 4 ounces of skimmed milk every two hours. For the past twenty-four hours the infant received 4 minims of paregoric every four hours, as from July 12 to July 17; was fed about two hours previous to test. 2:20, No. 14 (F.) catheter passed. Stomach empty. 2:35, catheter again passed into stomach. Same obstruction at cardia, which was passed; the sphincter was felt to grip the catheter when withdrawal was attempted. Could not pass the pylorus even with the aid of the "accessory method." Marked flow of gastric juice, giving a strong reaction for free hydrochloric acid (Congo).

3:40, catheter removed. Grip of cardia again clearly felt on withdrawal.

Epicrisis: Spasm of cardia and pylorus with marked gastric secretion.

September 15, Test 3: Weight, 4 pounds 15 ounces. Regurgitates much less. Has gained 7 ounces in four days. Still given paregoric in same doses. No. 16 (F.) catheter at once passed into duodenum and bile obtained. Child much quieter.

Here we have the record of a child which gave a history of vomiting during the second month of life, but when examined in the third month seemed normal, as far as our test is concerned, except for an excessive duodenal secretion. About two weeks later it began again to regurgitate persistently, and when tested for a second time gave unmistakable signs of cardiospasm and pylorospasm. The spastic condition developed under our observation. A third passage of the catheter, carried out a few days later, revealed the fact that the spasms had relaxed, and, coincident with this change in physical condition, a decided decrease in the regurgitation was noted, as well as a general improvement and gain in weight. This improvement took place subsequent to the passage of the catheter. However, in spite of the fact that it retained its food, it gradually sank, and died of marasmus about two weeks later. Duodenal feeding was not attempted, as there was no one at hand to carry it out satisfactorily.

It will be seen from a survey of these two cases, and I may add that other instances corroborate this view, that the duodenal catheter enables us to make an absolute diagnosis of pyloric spasm, and to sharply differentiate this condition from vomiting not spastic in nature. A single test generally has been sufficient to draw this distinction. However, if we merely fail to enter the duodenum in a case, we would certainly err to attribute this failure to spasm. But if we are sure of our technic, and have passed the catheter in numerous instances without difficulty, and then meet with a case offering persistent obstruction at the level of the pylorus, we are justified in attributing this hindrance to pyloric spasm. This can be our conclusion only if we finally overcome the obstruction. If we fail to enter the intestine, a single test must be considered insufficient, as the failure may be due to other causes, especially if our experience has not been wide. It is in such cases as I have detailed at length, in which the catheter is repulsed again and again at the same level, where we finally feel the resistance give way and the catheter pass, where there is a marked grip or tugging when the attempt to retract is made, that we may venture a diagnosis of spasm. It is not possible to differentiate between a simple spasm and a true stenosis of so mild a grade that it permits of the passage of a No. 15 (F.) catheter. However, if we are unable to enter the intestine after repeated attempts in the case of an infant showing symptoms of pyloric spasm or stenosis, the latter must be looked on as the more likely pathologic condition.

In most of our cases, in addition to the spasm of the pylorus, we have encountered a spasm of the cardia. That this functional disturbance has not been met with frequently by others can be accounted for only by a difference in technic. If a catheter is forcibly pushed down the esophagus into the stomach, a moderate increase of resistance of the cardiac sphincter will readily be overcome, and just as readily escape observation. However, if the catheter is inserted slowly and gently, it will be noted that there is frequently a definite resistance persistently met with at a point

which is about 15 or 16 cm. from the lips, whenever we attempt to enter the stomach. When a very soft rubber tube, such as the duodenal ball-tube, is employed, we find this obstruction intensified, so that, as in the two tests in Case 1, it becomes impossible to pass the cardia unless we relax this sphincter by having the baby swallow some liquid (accessory method). This interesting syndrome of pylorospasm adds weight to the argument of those who favor a neuropathic origin for this condition. In the case of an infant 7 weeks of age suffering from pylorospasm, it was found that frequently the food did not even enter the stomach, but was checked at the cardia and quickly regurgitated. In this case it was impossible to pass the soft tube into the stomach. When the catheter was inserted into the stomach this organ was found to be absolutely empty, a condition which in my experience is very exceptional.

Another observation which is interesting, and which has not, to my knowledge, been previously described, is an anomaly of secretion in the duodenum frequently accompanying pylorospasm. For some time it has been realized that many cases of spasm of the pylorus are accompanied by a marked increase in secretion of gastric juice. In the two cases which I have detailed this was true. In addition there was an increase in the secretion of the duodenal fluid. In the first case in Test 3, the first one in which I was able to enter the duodenum, 8 c. c. of fluid was aspirated within twenty-five minutes; in the next test 6 c. c. was obtained in thirty minutes, and in the seventh test, the only other in which the juice was collected, 8 c. c. of alkaline fluid was secreted in one hour. Comparative tests on other infants show unequivocally that this far exceeds the normal total of secretion for such short periods. The second case gives evidence that this hypersecretion, or "duodenal succorrhœa," as it may be termed, may persist even when the spasm is not present. It will be seen that in the first test of Case 2, which, it will be remembered, was undertaken when the baby was no longer vomiting, although gavage had been necessary about three weeks previously, a marked duodenal hypersecretion was noted; 13 c. c. of alkaline, greenish juice being obtained in thirty minutes. The fluids in all these instances were examined for the three pancreatic ferments, and found to contain them rather in excess, so that we must conclude that in cases of pylorospasm accompanied by gastric hypersecretion there is often also a pancreatic hypersecretion. It is well established that the gastric juice serves to stimulate the pancreatic juice, but, tempting as this explanation appears, it may be that both secretions have a common exciting cause.

I have intimated that some cases of pylorospasm are not accompanied by gastric hypersecretion. Most observers now distinguish between these two groups of cases. The case of the 7-weeks-old infant whom I referred to in connection with the obstinate cardiospasm, well illustrates this distinction. There was only one test performed, and this showed the stomach absolutely free of hydrochloric acid both at the beginning and at the end of the test. In this instance there was likewise a hypersecretion of duodenal fluid, instead of the succorrhœa which we encountered in the previous cases, so that, using also the pancreatic secretion as a criterion, we can differentiate two groups of cases.

USE OF THE CATHETER FOR THERAPY

In infants persistent vomiting may result in extreme inanition, or even death. We see this most frequently, perhaps, in cases of pyloric stenosis or of pylorospasm. In spite of all our efforts to relieve the spasm or to provide an appropriate food the vomiting persists. But in addition to this group of cases there are others less sharply defined, termed by some merely "persistent vomiting," for want of a more exact term, in which the infant dies of inanition, and the stomach is found normal at autopsy. Gittings[3] recently reported three such cases. Again, during or

[3] Gittings, J. C. Arch. Pediat., August, 1911, p. 661.

shortly following the acute period of some of the infectious diseases, such as pneumonia, vomiting may supervene and threaten or end the life of the weakened infant. I believe cases of this description are more frequent than is generally realized.

The main treatment of vomiting in infants, whatever its cause, may be summarized as dietetic, the selection of an appropriate food, or as mechanical, which includes the washing of the stomach, as well as feeding by means of the tube directly into the stomach. But even these methods are not always successful, and many infants succumb to marasmus or finally to some secondary infection. It is in these extreme instances in which the ordinary measures fail that I believe duodenal feeding has a place. The radiograph (Fig. 1) which I have had reproduced clearly demonstrates that this procedure is feasible. It shows us the catheter in the duodenum, and the bismuth-milk mixture in the various coils of the intestine. In adults Einhorn has employed duodenal feeding. For the purpose of feeding the catheter is introduced in the usual way, and is inserted to about the 40 cm. mark, if this can be accomplished without force. When the catheter has entered this distance I have refrained from moving it in and out, in order not to irritate the intestine. For the same reason, as well as on other physiologic grounds, I have always had the milk heated to about 100° F. It is probably also of advantage, judging from experiments *in vitro*, to have the milk peptonized, although I have many times given raw milk in this way without observing signs or symptoms of indigestion. The infant must be fed slowly. In some cases this precaution is forced on us, as the fluid enters the intestine with difficulty, and it even becomes necessary to force it onward by means of pressure, which is most readily carried out with the aid of the aspirating bulb. But where the fluid has a tendency to run rapidly through the funnel, which is attached to the catheter, the flow should be obstructed, and ten to twenty minutes should be consumed for the entire feeding. Otherwise we run the risk of inciting antiperistalsis by overdistending the gut, and of causing regurgitation of the fluid from the intestines into the stomach. Indeed, this happens to an inconsiderable extent whenever we introduce large quantities of liquid directly into the intestine. We may then find that some of it, perhaps an ounce, has found its way back to the stomach, and may later be regurgitated, although the bulk of the food has been satisfactorily retained.

I have had an opportunity of feeding a considerable number of infants by this method. In most cases I have done so but once or twice, because further opportunity did not present itself. In these instances the report has been that the food was entirely or almost entirely retained. Two urgent cases in which "duodenal gavage" was continued for some days seem worthy of a detailed report:

Case 3.—B. B. was admitted September 6 to Beth Israel Hospital, suffering from lobar pneumonia. The child was 10½ months of age, had been ill five days, and gave a history of cough, dyspnœa and fever. By September 11 the temperature was normal, falling by lysis. On September 7 the infant began to vomit its feeding, which consisted of 6 ounces of milk, 2 ounces of barley water, and 4 per cent. sugar every four hours. The vomiting increased in frequency, until by September 13 the child was retaining very little nourishment. Smaller quantities of food were given, lavage followed by gavage, sodium citrate, small doses of codein before feedings, but all to little purpose, and the child sank into a very critical condition. Accompanying a rapid loss of weight, the temperature dropped and remained subnormal. On the morning of September 15 I introduced 4 ounces of milk-mixture (three-quarters milk, one-quarter barley, sugar 2 per cent.) into the duodenum and it was retained. The day following I repeated this procedure with favorable result, so that regular duodenal feedings every four hours were begun. This was discontinued after four days, when it was found that the infant, in addition to having gained in weight, was able to retain most of its food when given directly by mouth. The child did not vomit frequently after this

date, and was discharged cured November 14. The feeding was carried out as above described.

This case showed the importance of introducing the food slowly, as whenever it was allowed to stream rapidly through the catheter marked gagging would result with a regurgitation of some of the milk. In fact, even if the procedure is performed with care there may be a reflux of a small quantity of milk into the stomach. For this reason it is better to limit the amount of feeding to from 1 to 4 ounces, according to the size of the child and the ease with which the milk can be successfully introduced. This is advisable also because these infants are generally suffering from a marked grade of inanition, and it is, therefore, unwise to allow them suddenly large quantities of food.

A second case in which the vomiting was more violent and the inanition more extreme is the following:

Case 4.—L. N., 18 months old, was admitted to Beth Israel Hospital, September 13, on account of enteritis. The intestinal disturbance yielded to "Eiweissmilch," and all seemed well, when on September 27 the child began to vomit and to give signs of nephritis. By September 29 the child had lost 1¾ pounds, its tongue was dry, its expression anxious; leucocyte count 21,000. Lavage was carried out, and small doses of codein given before feedings. By the day following it had lost 11 ounces more, that is, almost 2½ pounds in four days. Lavage had proved of very temporary value. The next day's weighing showed a loss of 2 ounces more. The child's condition was very serious, its eyes were sunken, its tongue dry, acetone was present in the urine, and all food was rejected whether given by spoon or by tube. The vomiting was markedly projectile in type; when the child lay on its back the milk was ejected in a vertical column of about 3 inches. Food in small quantity and in weak dilution was resorted to, but all was rejected, even small quantities of water.

October 1 duodenal feeding was attempted on two occasions with success, and the day following four feedings were given in this manner, and one feeding of 1 ounce by spoon. The duodenal feedings consisted of 6 ounces of equal parts of milk and water, with 3 per cent. sugar. This quantity, as I now realize, was unnecessarily large, and generally 1 to 2 ounces were rejected. The 1 ounce feeding by spoon was not retained. October 3, although the child had not gained, it looked better; by the next day it was found to have gained 2 ounces; and by October 6 an additional 6 ounces. The child looked much better, the urine no longer contained acetone, the pylorus seemed relaxed, so that feeding by spoon was once more attempted. The child now retained small quantities of food, and duodenal gavage was entirely abandoned. It was discharged cured from the hospital November 16.

The nature of this sudden attack of vomiting is obscure, but this is not of great moment from the viewpoint of the question of feeding. It was certainly spastic in nature, as proved both by the typically projectile character of the vomiting, and by the resistance which the pylorus offered to the catheter. This resistance became noticeably less after the first few introductions of the tube. It is difficult to judge at present whether this was the result of a dilatation and relaxation due to the instrumentation. It is possible that the passing of the catheter reduced the spasm of the pylorus. This seemed to be true in this instance, as well as in Case 2, described above. in reference to the diagnosis of spasm, and in cases observed by others following the passage of the catheter.

I cannot but feel that the tide was turned in the life of this infant by means of duodenal feeding. There was a sudden change for the better, and, although some of the food was regurgitated, as the quantity given was too large, the child gained in weight and strength. It should also be noted that we were able in a few days to abandon feeding by the catheter for regular feedings with the spoon.

It gives me pleasure to acknowledge my indebtedness to Dr. Caillé, and to Dr. Chapin, of the Post Graduate Hospital, for the privilege

accorded me of carrying out a large part of this work in their wards, and of thanking Miss Gertrude Fisher for her able assistance.

SUMMARY

In infants it is possible without difficulty to insert a soft rubber Nélaton catheter (No. 15 F.) past the pyloric sphincter and into the duodenum. The catheter is introduced in the same way as the ordinary stomach-tube, and, after some experience is acquired, the technic employed becomes almost as simple.

In principle the catheter differs from the duodenal tube previously described by the author mainly in that this instrument does not depend on gravity or peristalsis to direct it to the pylorus. The mere force of inserting it propels it along the natural path of the food to the pyloric opening. This fact not only enables it to be introduced readily and surely, but gives to it the additional advantage of a probe, a pyloric probe with which we may test the tonicity and irritability of this sphincter. Radiographs show that the catheter invariably, on entering the stomach, bends sharply to the left to reach the fundus, and that, therefore, the more vertical position of the stomach of the infant does not account for the ease with which the duodenum is entered.

It is probable that unknowingly others have entered the intestine by this method, and that in many instances reports as to the contents or the capacity of the stomach in infancy have been subjected to this source of error.

By means of the catheter we can readily diagnose pylorospasm, and differentiate it from vomiting due to other causes. In the case of spasm we meet with a persistent resistance encountered at the same point whenever we attempt to advance the catheter; this is frequently accompanied by irritability of the pylorus. The spasm may be felt to relax suddenly and enable us to enter the intestine.

Marked pyloric stenosis can be diagnosed from the failure to transgress the pylorus after repeated attempts. A mild degree of stenosis, so slight as to allow of the passage of the catheter, cannot be differentiated from simple spasm.

Cardiospasm frequently accompanies pylorospasm. This sign has been frequently overlooked, due to the too forcible insertion of the stomach tube. If a soft rubber tube, such as was originally used, is introduced into the esophagus, it may be found even impossible to enter the stomach. Frequently as the result of this spasm the food does not enter the stomach, being checked at the cardia.

Just as a marked gastric secretion frequently is associated with pylorospasm, so also is an increased duodenal secretion (duodenal succorrhœa). This secretion is found to contain protease, lipase and amylase to a marked degree, so that in this connection we may speak of a pancreatic hypersecretion or succorrhœa.

There are cases of cardiospasm and pylorospasm unaccompanied by increased gastric secretion. In a case of this kind there was likewise no pancreatic hypersecretion.

The catheter is of value in the therapy of pylorospasm. Its passage through the pylorus seems to relax the ring and in this way to diminish the vomiting. It would seem of advantage to test this method of dilating the pylorus, and to pass the catheter frequently in such cases.

Another form of therapy consists of duodenal feeding. Radiographs show that this is feasible. It should be reserved for such cases as do not retain food given by gavage, and the food should be given slowly and in not too large amounts. In cases of this kind it has been found of great value.

REPORT OF EXPERIMENTS ON STANDARDIZATION METHODS FOR BACTERIAL DIAGNOSIS OF DIPHTHERIA

M. A. Wilson

The experiments recorded here were made at the request of the chairman of the committee on standard methods for the bacterial diagnosis of diphtheria of the American Public Health Association.

Their purpose was to determine, first, whether the wire swab used in making throat cultures has an inhibitive effect upon the growth of the diphtheria bacillus; second, whether serum should be sent in diphtheria outfits or not; third, what types the majority of bacteriologists believe should be called positive for diagnosis and what for release.

Each of these questions was made the object of a special test which was carried out according to the plan outlined by the chairman of the committee.

1. Comparative test of wire and wood swabs—

The wire swabs employed in this work were those which are now used by the Board of Health of this city; the wood swabs were made in the same way, except that we used a slender wooden rod instead of the wire. Each swab was placed in a separate glass tube, plugged, and sterilized by dry heat. The swabs were inoculated by rubbing one wire and one wood over the infected area of a diphtheritic throat. Each swab was then replaced in its own sterile tube and left at room temperature for 18 hours or longer. At the end of that time each swab was rubbed over the surface of a tube of Löffler's blood serum, and those cultures were incubated for 24 hours at 37° C. Smears were made from that growth, and the cultures returned to the thermostat for another 24 hours. After inoculation on blood serum the swabs were placed in other tubes of the same medium and left in the dark at room temperature for 24 hours, after which they were placed in the thermostat and treated in the same manner as the tubes first inoculated.

We inoculated swabs from six positive and five negative throats, and in each case the cultures made from the wire were about the same as those from the wood, both as to the character of growth and the types of the organisms. In some cases we allowed the swabs to stand at room temperature in the dark from five to seven days before inoculation.

The results of this test are given in the following table:

TABLE I

Our No.	Diagnosis	Time Swab Stood at Room Temp. Before Inoculation	RESULTS			
			Wire		Wood	
			Growth	Klebs-Löffler Bacilli	Growth	Klebs-Löffler Bacilli
1	—	19 hrs.	+	—	+	—
4	—	7 days	+	—	+	—
6	—	48 hrs.	—		—	
8	—	3 days	±	±	±	±
9	—	48 hrs.	±	±	±	±
2	+	48 hrs.	—		—	
3	+	48 hrs.	+	+	+	+
5	+	5 days	+	+	+	+
7	+	24 hrs.	+	+	+	+
10	+	48 hrs.	+	+	+	±
11	+	24 hrs.	±	+	±	—

In reference to growth, + indicates abundant, ± scant, — no growth. In reference to the Klebs-Löffler bacilli, + represents numerous bacilli in the smear, ± moderate number, ± few, — none.

TABLE II

Patient	No. of Slide	Time Swab Stood at Room Temp. Before Inoculation	Condition of Swab at Time of Inoculation	Period of Incubation	Temp.	Result	*Types Found in Smears	
							Cultures from Dry Swab	Cultures from Moist Swab
V	1	Inoculated immediately		24 hrs.	37° C.	Abundant dry growth	**C**, **C**[1], C[2], **D**, **E**, D[2]	No culture
V	2	24 hrs. Semi-dark	Dry. Apparently no material	24 hrs.	37° C.	No growth		
V	3	2 days	Dry. Apparently no material	24 hrs.	37° C.	No growth		
V	4	3 days	Dry. Apparently no material	24 hrs.	37° C.	No growth		
W	1	Inoculated immediately		24 hrs.	37° C.	Good growth	No diphtheria bacilli	No culture
W	2	24 hrs. Complete dark	Dry. Apparently no material	24 hrs.	37° C.	Abundant growth on both cultures	C[2], **C**, **C**[1], **D**, D[2]	More bacilli than in dry **C**, **C**[2], **D**, D[2], **C**[1]
W	3	2 days Complete dark	Dry. Apparently no material	24 hrs.	37° C.	Abundant growth on both cultures	Very short forms D[2], E[2], **D**	Occasional E[2], C[2]
W	4	3 days Complete dark	Dry. Apparently no material	24 hrs.	37° C.	Scant dry growth, both cultures	**C**, **D**, **E**, C[2], D[2]	**C**, **D**, **E**, C[2], D[2]
X	1	Inoculated immediately		24 hrs.	37° C.	Good growth, both	**C**, **D**, **E**, C[2], D[2]	No culture
X	2	Complete dark 24 hrs.	Dry Yellow stain	24 hrs.	37° C.	Scant growth, dry culture No growth, moist culture	**C**, **D**, D[2], C[2]	
X	3	2 days Complete dark	Dry Yellow stain	24 hrs.	37° C.	Scant growth, both	**C**, **D**, C[2], D[2]	**C**, **D**, C[2], D[2]
X	4	3 days Complete dark	Dry Yellow stain	24 hrs.	37° C.	No growth		

XX	1	Inoculated immediately		24 hrs.	37° C.	Good growth	**C**, C^2, **D**, D^2	No culture
XX	2	24 hrs. Complete dark	Dry Reddish stain	24 hrs.	37° C.	Dry culture = Pyocyaneus Moist, abundant dry growth	**C**, C^2, **D**, D^2, pyocyaneus	**C**, C^2, **D**, D^2
XX	3	2 days Complete dark	Dry Reddish stain	24 hrs.	37° C.	Scant, both	**C**, **D**, **E**, C^2, D^2, E^2	**C**, **D**, **E**, C^2, D^2, E^2
XX	4	3 days Complete dark	Yellow stain	24 hrs.	37° C.	Abundant growth, both	Few groups of E^2, D^2	Few **C**, C^1, D^2, E^2
Y	1	Inoculated immediately.		24 hrs.	37° C.	Good growth	**C**, C^2, **D**, D^2	No culture
Y	2	24 hrs. Complete dark	Dry Very faint creamy stain	24 hrs.	37° C.	No growth		
Y	3	2 days Complete dark	Dry Very faint creamy stain	24 hrs.	37° C.	No growth		
Y	4	3 days Complete dark	Dry No stain	24 hrs.	37° C.	No growth		
Z	1	Inoculated immediately		24 hrs.	37° C.	Good growth	D^2, E^2, **C**, C^1	No culture
Z	2	24 hrs. Complete dark	Dry Faint creamy stain	24 hrs.	37° C.	Abundant dry growth, both	Occasional C^1	Occasional D^2
Z	3	2 days Complete dark	Dry Faint creamy stain	24 hrs.	37° C.	Good growth, both	No diphtheria bacilli	No diphtheria bacilli
Z	4	3 days	Dry Faint creamy stain	24 hrs.	37° C.	No growth		

*The plain letters C, D, E, designate diagnostic types, according to Westbrook's classification. The letters with exponent designate non-diagnostic types according to Westbrook's classification.

TABLE III

Diagnosis Cultures

Original Throat Culture			Subculture on Special Serum			
Patient	Diagnosis	Types Upon Which Diagnosis Was Made	Growth	Temp.	No. of Slide	Types
A	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	3	**C**, **D**, **E**, C², D², E².
C	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	4	**C**, **D**, **E**, D², E², C².
D	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	1	**C**, **D**, **E**, D², E², C².
F	+	**C**, **D**, **E**, C², D², E², B², **B**	15 hrs.	36.5° C.	3	**C**, **D**, **E**, **A**, C², D², E², **B**, B².
G	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	3	**C**, **A**, **D**, **E**, C², D², E².
H	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	3	**C**, **D**, **E**, C², D², E².
J	+	**B**, **C**, **D**, **E**, **B**¹, C², D², E²	15 hrs.	36.5° C.	3	**B**, **C**, **D**, **E**, **B**¹, **C**¹, C², D², E².
K	+	**C**, **D**, **E**	15 hrs.	36.5° C.	3	**C**, **D**, **E**, C², D², E².
L	+	**A**, A², **B**, **B**¹, D², E²	15 hrs.	36.5° C.	3	**C**, **D**, **E**, **B**, C², B², D², E².
M	+	**C**, **B**, B², C²	15 hrs.	36.5° C.	2	**C**, **D**, **E**, **B**, B².
N	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	2	**C**, **D**, **E**, C², D², E².
O	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	4	**C**, **D**, **E**, C², D², E².
P	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	3	**C**, **D**, **E**, C², D², E².
R	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	3	**C**, **D**, **E**, C², D², E².
S	+	**C**, **D**, **E**, C², D², E²	15 hrs.	36.5° C.	3	**C**, **D**, **E**, C², D², E².

TABLE IV

Release Cultures

Original Throat Culture			Subculture on Special Serum				
Patient	Release Diagnosis	Types	Growth	Temp.	Humidity	No. of Slide	Types
θ	+	B^2, D^2, E^2, **E**.....	15 hrs.	36.5° C.	43° C.	2	Few B^2, E^2, **E**, **D**.
ξ	+	Few Atypical **C**, **D**, **E**.....	15 hrs.	36.5° C.	43° C.	1	Large bacilli with granules. Some forms approaching **C** and **D**.
π	+	D^2, E^2, C^2.....	15 hrs.	36.5° C.	not taken	3	D^2, E^2, **D**.
Σ	+	Few **D**, D^2, **E**, E^2.....	15 hrs.	36.5° C.	43° C.	1	D^2, E^2, occasional **C**.
ϕ	+	D^2, E^2, many.....	15 hrs.	36.5° C.	43° C.	1	Few E^2, D^2.
ψ	+	Few **E**, E^2.....	15 hrs.	36.5° C.	not taken	1	Few **E**, E^2.

2. Should serum be sent in diphtheria outfits?

"A matter of great interest to state laboratories is the question as to whether serum should be sent in diphtheria outfits or not. There are some difficulties in the way, but these can be overcome if experience shows that it is desirable to have the serum inoculated at the bedside."

The above paragraph was the preface to the request that we should test the viability of organisms which had been allowed to stand several days on the dry swab before cultures were made.

In this work we took four swabs from the throat of a diphtheria patient. Inoculated one immediately on blood serum. The others were kept in sterile test-tubes in the dark at room temperature for one, two and three days, respectively, and finally inoculated on blood serum. Each culture was incubated for 24 hours, and at the end of that time smears were made.

This experiment was repeated on swabs from five different patients with slightly varied results, which are given in the second table.

3. Types of diphtheria bacilli—

In the summer of 1909 we made a study of the types of diphtheria bacilli found in throat cultures, and several months later were asked to do some supplementary work upon that subject.

In the first experiment the committee provided special serum upon which we made subcultures from fifteen positive diagnosis cultures. These were incubated for 15 hours at 36.5° C. We followed the same procedure with ten release cultures. By *release* cultures we mean negative cultures from throats which had been positive upon admission. From these cultures on the special serum we made slides at the end of 15 hours, stained them with methylene blue, and recorded the types upon which the diagnosis was made. We followed the Westbrook-McDaniels classification in the identification of our types.

The original throat cultures from which we made our subcultures were sent to us from the Diagnosis Laboratory. In tabulating results we gave the types found in the original, as well as in the subculture. In a few cases the types in the subculture varied slightly from those in the original, as shown in Tables III and IV.

Tables V and VI give the result of the supplementary work on types of bacilli. In this study we made no subcultures, but recorded the types found in the original throat cultures sent from the Diagnosis Laboratory.

TABLE V

DIAGNOSIS CULTURES

Patient	Diagnosis	Types Found (Diagnosis Made from whole Picture)
A	+	**C, D, E**, C², D², E², **C¹, A**, A²
B	+	**C**, C², **D, E, F**, D², E²
C	+	**D, E, C**, C², D², **F**
D	+	**C, D, E, F**, D², **C¹**, C²
E	+	Slender **C**, D², E², **A**, C², **C¹**
F	+	**C, D, E, F, G**, C², D²
G	+	**D, E, C**, C², D², E², **F, G**
H	+	**C, B, D, E, C¹, B¹**, C², D²
I	+	**C, D, E, F, C¹**, C², D², **A**
J	+	Slender **C, D, E, F**, C², D², E²

TABLE VI

RELEASE CULTURES

Patient	Diagnosis	Types
M	+	No diphtheria bacilli
N	+	Few atypical D^2, E^2
O	+	No diphtheria bacilli
P	+	Few **C^1**, C^2, D^2, **E**
Q	+	Few C^2, D^2
R	+	Small group E^2
S	+	No diphtheria bacilli
U	+	No diphtheria bacilli
V	+	No diphtheria bacilli

ATYPICAL TYPES

Upon the special serum we made subcultures from five throat cultures which were suspicious, showing diphtheria-like bacilli. These atypical types were isolated in pure culture and their virulence tested. We also tested their acidity in dextrose and saccharose broth after ten days' growth. These results are given in Table VII.

TABLE VII

Patient	Diagnosis	Acidity		Original Acidity of Medium	Virulence
		Dextrose	Saccharose		
B	Atypical.	5.4% acid..	7% acid....	1.2% acid..	Death 48 hrs.
I	Atypical.	5.6% acid..	2.4% acid..	1.2% acid..	Death 48 hrs.
U	Atypical.	6.0% acid..	Neutral...	1.2% acid..	Death 48 hrs.
V	Atypical.	5.4% acid..	8% acid....	1.2% acid..	Death 42 hrs.
W	Atypical.	5.6% acid..	6% acid...	1.2% acid..	Nonvirulent
G	Atypical pseudo.	not tested..	.....	.....	

RÉSUMÉ

This work has emphasized the following points:

1. A second culture generation on a favorable medium (for diphtheria bacilli) may show many more typical bacilli in a mixed culture from a patient than the first culture generation.

Where there are *many* diphtheria bacilli in the first culture generation the above fact would not change the original diagnosis, but where there are few, they may not be found in the first culture, or may grow atypically, so that positive diagnosis cannot be made, while in a second culture generation they may spread and grow quite typically. This hap-

pens to be the case in a number of our release and atypical cultures. On the other hand, some of our second generations were overgrown by contaminating organisms. Therefore, our diagnoses sent to the committee do not agree in every case with our original diagnosis.

2. This emphasizes the fact that a uniform favorable culture medium should be used and is a point against the utility of this work.

3. In routine diagnosis of diphtheria one can never be positive that there are not some diphtheria bacilli in the culture examined by smear alone. We had this strikingly illustrated in a culture we classed as an atypical pseudo-diphtheria G. It was atypical in that it contained more forms than usual, which were rather brilliantly stained and slightly irregular in size and shape. Upon isolating by the serum-broth method apparently only pseudo-diphtheria bacilli were obtained, but the animal inoculated died within 48 hours of typical diphtheria poisoning, and a typical diphtheria bacillus was obtained from the point of inoculation.

Going back to the original throat culture, serum-broth cultures were again made and from these plates and numerous fishings were made, and one out of the lot proved to be typical pseudo, while the others were all typical diphtheria. The same examination of a special serum culture gave one typical diphtheria culture among several pseudo cultures. These cultures are undergoing further study.

4. In regard to using Westbrook's types, we still think that no special types should be used in making the diagnosis, but that the composite picture should be considered for the following reasons:

1. Pure cultures of the pseudo-diphtheria bacillus may contain an occasional D and E.

2. Pure cultures of B. pyocyaneus may contain an occasional C, D, and F.

3. Pure culture of B. alvei may show thick B's, C's and D's.

4. If the medium is a little dry, or in the presence of certain contaminating bacteria, especially if there are *few* diphtheria bacilli, the latter may grow quite atypically, *e. g.*, show exclusively types approaching the D^2 types of Westbrook; or produce forms that Westbrook has not depicted at all—as what has been called the "tailed" form, a rather intensely-staining body—presumably a diffused granule—with a faintly staining, pointed projection. This form occurs quite frequently.

If one had to choose Westbrook's types, the following are considered by us to be virulent diphtheria bacilli:

All the granular forms except G; of the barred forms, A, C, and possibly E, though this last is really a typical xerosis bacillus.

COMPARATIVE TOXIN PRODUCTION IN VARIOUS STRAINS OF DIPHTHERIA BACILLI

JANE L. BERRY, M. D., ASSISTED BY LOUISA P. BLACKBURN

The diphtheria antitoxin made by the New York Health Department is all produced by means of the action of a single strain of diphtheria organisms known in the laboratory as No. 8.

The organism used was obtained originally in the year 1895 in the course of ordinary routine culture examinations from the throat of a child who had so slight an attack of diphtheria that it was at first diagnosed as tonsilitis. The animal tests which were being made with many strains at that time in the search for a good toxin producer showed this strain to be more active than any of those tried, and it was therefore selected for use in the preparation of toxin for the inoculation of horses. It has been constantly in use since that time for this purpose, has been kept growing in neutral broth, transferred about every third day, and has shown a remarkable persistence in vigor and toxin production. While this has fluctuated within wide limits at times, yet the careful studies made by Dr. A. W. Williams soon after isolation (Jour. Med. Res., June, 1902), and confirmed by all subsequent work with the strain, show this fluctuation to be due to some alteration in the culture medium rather than to any inherent change in the organism itself, for a more careful preparation of the broth is always followed by a return to the same high toxin production, 1-400 to 1-500 c. c., and occasionally as high as 1-800 to 1-1,000, proving fatal within 4 days to medium sized guinea pigs.

Morphologically the organism has remained unchanged during this long period of growth in the laboratory, except that the rods are somewhat longer than when first isolated. In agar it has always produced very characteristic spreading colonies, and grows in broth with a typical heavy pellicle lying above a clear fluid.

Many comparative tests in the laboratory have always failed to find a culture stronger than No. 8 in toxin production, or even another equally active, but because of Dr. Park's desire to be thus provided for the important work of toxin production it was decided to make another attempt, taking a large number of strains from different sources, and testing for toxin production soon after isolation, and again after a long period of cultivation; these results to be compared with those given by No. 8, grown under exactly similar conditions.

For this purpose 100 diphtheria cultures were obtained without selection, 25 being taken from the throats of diphtheria patients in the Willard Parker Hospital and 75 from the cultures sent for diagnosis from the city to the Health Department laboratories. In the private patients membrane was stated to be present on the throat in 67 cases, not mentioned in 7 cases, absent in one case. Membrane was present on the throats of all the hospital cases, and generally in considerable amount, as nearly all were cases of a severe type. All strains without exception showed typical diphtheria bacilli, that is to say, forms corresponding to Westbrook's types A, C and D. In most strains these were the predominating types, and in many almost the only forms present. Such strains gave from the beginning a vigorous growth in broth, with typical pellicle formation, and on agar characteristic spreading colonies. The other strains showed, besides the typical organisms, many smaller, more evenly straining forms on Löffler's blood serum, and in most cases these strains grew with difficulty at first in broth, with pellicle either slight or absent, and on agar showed somewhat small, dense colonies, with but little tendency toward spreading.[1]

[1] The individual organisms of these strains grown on agar were not studied, but they probably are strains producing large segmenting forms on agar as described by Williams.

All strains were carried on in neutral broth cultures at 37° C., transferred every third day, with frequent platings to insure purity. In those strains that were at first less characteristic there was an increasing tendency in later cultures toward better growth, with pellicle production in broth and characteristic colony formation on agar, but in a few cases strains remained somewhat atypical throughout in these media, although never failing to produce characteristic bacilli when grown on Löffler's serum tubes.

After an interval long enough to allow the organisms to become accustomed to growth in an artificial medium, generally at about the sixth to the twentieth transfer, sometimes later, all strains were tested for toxin production.

Broth cultures grown in the incubator for 8 days were examined by means of transfer to agar plates and blood serum tubes, carbolic acid was added in 1 per cent. amount, and after two days' interval subcutaneous inoculations were made into medium sized guinea pigs. The dose ranged from 1-10 to 1-200 c. c., according to culture conditions and size of animals, while in a few of the less toxic strains as much as 3 c. c. was given in later tests. At the same time a corresponding test was made with the same quantity of a culture of No. 8 grown in the same broth and under the same conditions.

The result showed that most of the strains, including some of those having the most characteristic cultural properties, produced much less toxin than the stock culture No. 8. Many failed to kill in less than 1-10 c. c. and a few were not fatal even in larger amounts, while death, when it did occur, was generally much later than in the control animals inoculated with No. 8.

With a few strains in these early tests the results were at first more nearly parallel, guinea pigs injected with 1-10 to 1-50 c. c. dying as soon as, and in one or two cases even a little earlier than, the corresponding control animal, but when successively smaller doses were given in all cases there was a lengthening interval, the new strain always showing less toxicity than the stock culture

All strains were now again carried on for some time in broth and after a varying number of transfers, generally from 30 to 40, and in those first isolated from 80 to 100, or more, the strains were again incubated for toxin production, together with No. 8, and tested as before.

The results were even more disappointing than in the earlier series as to finding a better culture than No. 8, or even a second equally active toxin producer. In some cases the amount of toxin production was comparatively unchanged, but the majority of the strains, among them some of those formerly giving the best results, now fell still further behind No. 8.

The toxin from five strains failed to kill in less than 3 c. c. and that from nine others was not fatal even in this amount, but this result was evidently largely due to the lack of ability in these organisms to grow well in the broth used, for when, at Dr. Williams' suggestion, they were cultivated for several transfers in ascitic growth and then tested for their virulence in every case but one death followed the inoculation of 1-10 c. c. of the living culture. This one was classed with the non-virulent and hence non-toxin-producing organisms. The amount of toxin production in ascitic broth was not tested. Three other strains grew with such difficulty in ordinary broth that no satisfactory toxin tests could be made with these cultures, but, grown in ascitic broth and tested for virulence, these strains also proved fully active in doses of 1-10 c. c. These results correspond exactly with those obtained by Dr. Williams in 1902. The best results in toxin production obtained with the entire series may be summarized as follows:

TABLE I

3 strains killed in 1/200 c. c.

11	"	"	"	1/100	"
25	"	"	"	1/50	"
6	"	"	"	1/30	"
11	"	"	"	1/20	"
27	"	"	"	1/10	"

The following table shows the relation between toxin production and virulence in the 17 strains producing little or no toxin in ordinary broth, and tested for virulence in ascitic broth:

TABLE II

	TOXIN Neutral Broth		VIRULENCE Ascitic Broth	
Strain	Dose	Result	Dose	Result
8	3 c. c.	Lived	1/10 c. c.	x 3 days
31	3 c. c.	x 2 days	1/10 c. c.	x 2 days
32	3 c. c.	Lived	1/10 c. c.	Lived
43	3 c. c.	Lived	1/10 c. c.	x 3 days
44	3 c. c.	Lived	1/10 c. c.	x 3 days
59	3 c. c.	x 2 days	1/10 c. c.	x 3 days
61	3 c. c.	Lived	1/10 c. c.	x 3 days
63	3 c. c.	x 2 days	1/10 c. c.	x 4 days
*66	.....	.....	1/10 c. c.	x 1 day
85	3 c. c.	x 2 days	1/10 c. c.	x 3 days
*90	.....	.....	1/10 c. c.	x 3 days
94	3 c. c.	Lived	1/10 c. c.	x 3 days
95	3 c. c.	Lived	1/10 c. c.	x 2 days
96	3 c. c.	Lived	1/10 c. c.	x 2 days
97	3 c. c.	x 2 days	1/10 c. c.	x 3 days
98	3 c. c.	Lived	1/10 c. c.	x 2 days
*99	.....	.....	1/10 c. c.	x 3 days

*No satisfactory toxin tests in neutral broth owing to poor growth.

The 17 virulence tests with ascitic broth are given in detail, contrasted with the toxin tests of the same strains in broth without ascitic fluid, as illustrative of the extreme sensitiveness of the diphtheria bacillus to slight changes in its nutrient medium. The overlooking of this fact is probably accountable for many of the conflicting statements in the reports of different investigators. Further work in this direction might have brought out finer comparative differences.

The result of this series of tests, carried on through so many transfers, and with so large a number of cultures, emphasizes strongly the unusual vigor and toxin production of the No. 8 laboratory strain and the retention of these qualities for so long a time in an artificial medium is a striking illustration of the relative stability of the diphtheria organism.

NON-VARIABILITY IN TYPE OF DIPHTHERIA BACILLI

JANE L. BERRY, M. D., AND EDWIN J. BANZHAF, PHAR. D.

In the Journal of Infectious Diseases, October, 1908, Goodman reported a series of tests with diphtheria bacilli by which he claimed to have established the fact of a natural process of selection among diphtheria organisms, and stated that by taking advantage of this he had been able to obtain from a strain showing medium acid production in glucose broth a diverging series, leading to two opposite extremes with regard to acid production.

Starting with a single colony from a case of clinical diphtheria, he inoculated a series of tubes of sugar-free infusion broth containing 1 per cent. dextrose, incubated for three days at 37° and then titrated to determine the final acidity. From the cultures showing the highest and lowest acidity 15 tubes of glucose broth each were inoculated, incubated and titrated as before. In this manner the two strains, called the high and the low respectively, were now carried through 36 transfers, the culture showing the maximum acidity being the only one selected each time to carry on in the high series, and the one with the minimum acidity in the low series, plating weekly to avoid contamination.

While at first some irregularity was noticed, Goodman states that he soon found a gradually increasing variation in fermenting power between his high and low strains, until at the end of his experiment he had as wide a divergence as between a diphtheria and a pseudo-diphtheria strain, leading on the one hand to a very high acid-producing organism and on the other to an organism producing alkali in glucose broth.

On the basis of this work he suggests that the division into several distinct species is probably based on a misconception, and that all diphtheria-like organisms are probably only variants of the one species.

Winslow and Walker (Journal of Infectious Diseases, February, 1909), taking up the same problem, consider that an important factor in Goodman's work was probably the progressive effect of environment, the organism being exposed for transfer after transfer to high and low acidities respectively, and therefore likely to gain or lose tolerance to the acid reaction. They planned by excluding this factor to deal only with spontaneous variations. Selecting strains of the two types A and B of the paratyphoid bacillus of Schottmüller, they plated and fished from each 100 colonies into agar tubes.

As soon as these showed distinct growth each was transferred to a tube of 1 per cent. glucose broth, grown 72 hours at 20° and titrated. Next, going back to the original agar slants, those fishings which had shown the highest and the lowest acidities in glucose broth were selected to carry on for the high and low types of each organism, and from each of these, plated out, a series of glucose tubes were inoculated and carried on as before.

The conclusion of this experiment showed quite opposite results to those reported by Goodman with the diphtheria bacillus. With an occasional overlapping in acid production, each series varied around a distinct center, and in each there was a complete reversion to the original mean of the type, no apparent modification of character having been produced by the above process. The entire 538 cultures of the two paratyphoid types fall between the limits of 1.15 and 1.95 acid, yet in this narrow range are evident two distinct modes corresponding to the two apparently distinct paratyphoid types.

Our work along these lines was carried out with two strains of diphtheria organisms selected from among the 100 diphtheria cultures which had been previously obtained from the throats of diphtheria patients for use in a different study, and which had all been cultivated for many transfers on artificial media. Each of these 100 strains was transferred to glucose broth, grown for 3 days in the incubator, and then titrated with

phenolphthalein as an indicator, the results being tabulated according to acid production. A second test of the 100 strains was made in the same manner, and two strains were then selected for further experiment. One of these, known as No. 71, while always showing entirely typical organisms on Löffler blood serum, grew with difficulty in broth when first isolated, producing no pellicle until after long cultivation, and showing dark, coarsely granular, atypical colonies on agar. This organism produced but little toxin in broth, requiring 1-10 c. c. to kill a small guinea pig in 4 days, and the preliminary titrations showed but little acid produced in glucose broth. The other strain, No. 51, was active in all respects, growing vigorously and typically from the beginning in all media, producing sufficient toxin in broth to kill guinea pigs in 2 days with 1-100 c. c. and 6 days with 1-200 c. c., and showing a high acid production in glucose broth.

It was thought that by starting with these two strains, already at opposite poles of the scale of ordinary variation, there would be the most favorable opportunity offered for the development of Goodman's natural selection process.

The glucose broth used in the experiment was made in the chemical laboratory under Dr. Banzhaf's direct supervision, and the titrations were carried out by Miss Fisher and Miss Stryker under Dr. Banzhaf's direction.

The broth was made as follows: The clean muscle meat from young veal was ground up. Tap water was added (1 liter water to 1 pound of ground up meat). An 18-hour colon culture in broth was added. This mixture was allowed to ferment about 20 hours at 22° C. It was then digested for 2 hours at about 55° C. Boiled for 15 minutes and the meat strained off. Two per cent. of peptone and ½ per cent. of salt was added. The medium was then titrated, using phenolphthalein as an indicator. Sufficient normal sodium hydroxide was added to adjust the acidity to ½ per cent. normal acid. Boiled for 15 minutes and filtered clear.

The titrations were carried out in the original tubes of glucose broth culture. Each tube contained 10 c. c. of the medium. Phenolphthalein was used as the indicator. Decinormal sodium hydroxide was used to determine the acidity. The titration was carried on against a white background until a faint pink color appeared in the medium. With practice the faint pink color in the medium can easily be distinguished.

The two strains selected for the experiment were plated out on agar, and 100 colonies from each strain were fished onto the surface of agar slants, over which 1-10 c. c. of ascitic fluid had previously been dropped from a pipette. The fishing needle was washed off in the water of condensation and ascitic fluid in the bottom of the tube, and this was then floated up over the surface of the agar. By this method a good general growth was produced over the surface of the slant, thus avoiding the usual irregular growth from fishings.

The agar cultures after 24 hours in the incubator were washed off with 1 c. c. of ascitic fluid into tubes of glucose broth, which were grown in the incubator for 3 days and then titrated as above described.

The result of these titrations showed a different ratio of acid production to that given by the two strains in the preliminary tests, but, as the two trials then made had agreed in showing high acidity in No. 51 and a low grade in No. 77, and because of their suitability in other respects, it was decided to go on with the experiment as planned, using these two strains.

Going back to the original agar tubes, which had been returned to the incubator and again showed a good growth, the three fishings were selected which had shown the highest acid production, and also the three showing the lowest acid production in each strain. These were all plated out, and from 16 to 20 or more colonies of each kind fished into tubes of agar slants, plus ascitic fluid, as before, thus averaging about 60 fishings each for the new high and new low series in each strain. These were now transferred to glucose broth, grown and titrated as before, and again the three highest and three lowest selected for further use.

By proceeding in this way it was hoped to eliminate mere accidental variations, and to give the fullest opportunity for the influence of natural selection. No. 77 was thus carried through eight and No. 51 through nine series of cultures and titrations, with occasional sets missing, owing to breakage or contamination. The results are given in the accompanying table, where the figures show the number of fishings giving the same amount of acid in each set, and the connecting lines show the source of the successive groups. Results practically identical in both strains.

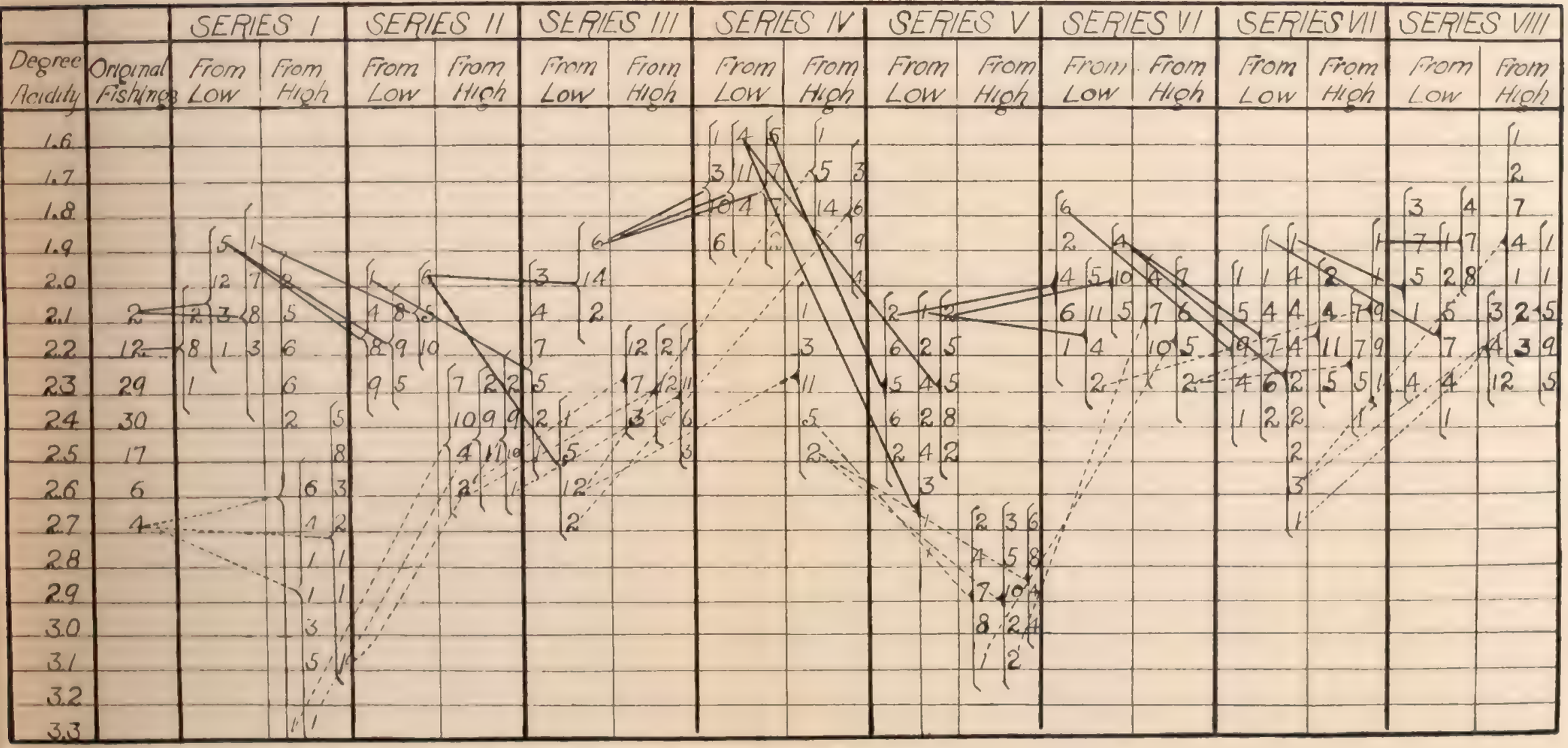
It will be seen that throughout this experiment there is no evidence whatever of the progressive development described by Goodman. Although there seemed at first some indication of a tendency toward division on the basis of acid production, this soon disappeared so completely that the most striking feature of the whole experiment with both strains was the tendency shown by the various fishings not to diverge, but to approach each other in the degree of acid production. The irregularity was marked, so much so that conditions were sometimes reversed between two series, the low of one furnishing the high of the next succeeding series, and vice versa, but at the end of the experiment all fishings are seen to be grouped within a very narrow range of acid production.

It may be said that results might have been different had we carried the experiment farther, but with the large number of sub-strains used at each transfer, together with the improved technic suggested by Winslow and Walker, it seemed to us that, if such a tendency exists in the diphtheria bacillus as described by Goodman, there should have been at least some indication of it in our series, amounting to over 1,000 titrations for No. 77 and over 1,100 titrations for No. 51.

We are brought, therefore, to conclusions exactly opposite to those of Goodman, and the result of this experiment seems to us to furnish another proof, in addition to those already demonstrated at this laboratory, of the fixed characteristics and non-variable nature of the diphtheria organism.

TABLE

TITRATION GLUCOSE BROTH CULTURES (NO 77)



THE RELATIVE IMPORTANCE OF THE BOVINE AND HUMAN TYPES OF TUBERCLE BACILLI IN THE DIFFERENT FORMS OF HUMAN TUBERCULOSIS*

Wm. H. Park, M. D., Charles Krumwiede, Jr., M. D., Bertha Van H. Anthony, M. D., Marie Grund, M. D., assisted by Louisa P. Blackburn

The results of the examination of various cases as given in Volume V showed conclusively that infection with bovine tubercle bacilli is essentially a disease of children, and a serious menace to life in infants. Because of this it was decided to examine additional material, especially from fatal cases of tuberculosis in children. The additional cases have given results closely approximating those already published and, therefore, serve to verify the reliability of these figures.

No further details are given of technical procedures or methods of differentiation. These have been fully discussed and there is nothing to add. The only point that needs mention and cannot be too often reiterated is that correct cultural differentiation depends largely on suitable culture media. Even with long experience, one finds irregularities creeping in, *viz.*, minimum growth of human viruses on some batches of media, or irregularities in individual tubes planted from the same material. We have prepared egg media in numerous ways and with very varying reactions and have been unable to find anything that accounts for these variations. In the routine examination of a series of cases these irregularities are quickly noted and controlled. They would be especially dangerous in the cultural differentiation of isolated cases if rabbits were not used to control the results.

The above remarks must not be interpreted as a criticism of the egg medium, for there is no doubt that it is very nearly a perfect medium for isolation and cultural differentiation and shows a minimum of variability.

The study of the Smith reaction has been continued and the complete results are given separately. (See page 116.)

Differentiation in the following additional cases has in some instances been on cultural characteristics alone. The bovine viruses have, however, been fully tested in rabbits. The cultures were not tested further than for differentiation in most instances, and in the tables are given as bovine, or human in this case, the grouping under the type not having been worked out.

*The following report gives additional data, on the examination of added cases. In Volume V the technical details and conclusions have been given in complete form. In Volume IV the case histories up to Number 500 are given. The following gives the remainder of the case histories. These details were considered too voluminous to include in the reports as published.

TABULATION* OF ADDITIONAL CASES EXAMINED

Diagnosis of Cases Examined	Adults 16 yrs. and over		Children 5 to 15 yrs.		Children under 5 yrs.		Notes
	H.	B.	H.	B.	H.	B.	
Pulmonary Tuberculosis	3	..	..	..	2	..	
Tuberculous Adenitis, Cervical	..	..	..	..	..	1	Case from Babies' Hospital.
Abdominal Tuberculosis	..	..	..	..	1	..	
Generalized Tuberculosis, Alimentary Origin	..	..	..	..	..	1	
Generalized Tuberculosis	..	..	..	..	6	..	
Generalized Tuberculosis, Including Meninges	1	..	..	..	7	..	
Tubercular Meningitis	1	..	1	..	12	1	
Tuberculosis of Bones and Joints	..	..	..	..	1	..	
Genito-Urinary Tuberculosis	3	..	..	..	..	..	
Tuberculosis of Skin	1	..	..	..	..	..	
Totals	9	..	1	..	29	3	Total Cases, 42.

Including these cases in the tabulations already published gives the following tabulation of cases examined.

*For method of tabulation see Vol. 5.

FINAL TABULATION OF CASES EXAMINED

Diagnosis of Cases Examined	Adults 16 yrs. and Over		Children 5 to 16 yrs.		Children Under 5 yrs.		Notes
	H.	B.	H.	B.	H.	B.	
Pulmonary Tuberculosis	281	..	8	..	7	..	Clinical diagnosis only known and therefore no positive details as to the extent of lesions elsewhere.
Tuberculous Adenitis, Inguinal and Axillary	1	..	4	..	..	..	(See next.)
Tuberculous Adenitis, Cervical	9	..	19	8	6	13	In two cases cultures were from axillary nodes but the primary focus was cervical. Another case died shortly afterward with pulmonary tuberculosis.
Abdominal Tuberculosis	1	..	1	1	1	3	Milk supply of one child subsequently examined. Tubercle bacilli isolated.
Generalized Tuberculosis, Alimentary Origin	..	..	..	..	1	2	Only three cases given under this heading. Many of the cases in the following subdivisions showed marked intestinal lesions and some possibly were of alimentary origin.
Generalized Tuberculosis	2	..	1	..	18	4	One bovine case had tuberculous osteomyelitis of the metatarsal bone.
Generalized Tuberculosis, including Meninges	1	..	..	..	25	1	

FINAL TABULATION OF CASES EXAMINED (Continued)

Diagnosis of Cases Examined	Adults 16 yrs. and Over		Children 5 to 16 yrs.		Children Under 5 yrs.		Notes
	H.	B.	H.	B.	H.	B.	
Tubercular Meningitis	1	..	2	..	26	2	No autopsy. Extent of lesions elsewhere unknown.
Tuberculosis of Bones and Joints	1	..	10	..	7	..	
Genito-Urinary Tuberculosis	6	1	1	..	..	..	The adult bovine case was tuberculosis of kidney. Removal of kidney. Complete recovery.
Tuberculosis of Skin	1	..	..	..	..	..	
Tuberculosis of Abscess	1	..	..	..	..	..	Possibly primary in bone.
Totals	305	1	46	9	91	25	

Double infection. Both types isolated. Generalized tuberculosis including meninges, 13 months. Mesenteric nodes gave human type. Meningeal fluid gave bovine type. Total cases, 478.

The additional cases necessitate some change in the mortality tabulations given in Volume V. As it is noted, there is a slight lowering of the percentages in the Babies' Hospital series and also in the cases considered as a whole.

TOTAL FATAL CASES IN CHILDREN

Diagnosis	Children 5 to 16 yrs.		Children Under 5 yrs.		Notes
	H.	B.	H.	B.	
Pulmonary Tuberculosis	..	..	7	..	One case included, probably fatal. Data incomplete.
Tuberculous Adenititis..	1	..	..	..	Other cases recovered as far as is known.
Abdominal Tuberculosis	..	..	..	3	Three other cases, one bovine and two human, were operative cases, with recovery, as far as known.
Generalized Tuberculosis.....	1	..	19	4	Two other bovine cases died directly of exanthemata with complications.
Generalized Tuberculosis, including meninges	..	..	25	2	One case gave both types of bacilli; included under bovine as this type caused the meningitis.
Tubercular Meningitis..	2	..	26	2	
Totals.....	4	..	77	11	Or 12½ per cent. due to bovine type under 5 yrs.

FATAL CASES—BABIES' HOSPITAL

Diagnosis	Children 5 to 16 yrs.		Children Under 5 yrs.		Notes
	H.	B.	H.	B.	
Pulmonary Tuberculosis	..	..	6	..	One case included probably fatal. Data incomplete.
Generalized Tuberculosis	1	..	14	2	
Generalized Tuberculosis, Including Meninges	..	..	21	1	One case gave both types of bacilli; included under bovine, as this type caused the meningitis.
Tubercular Meningitis	..	..	18	1	
Totals	1	..	59	4	Or $6\frac{1}{3}$ per cent. due to bovine type, under 5 yrs.

No additional cases from the Foundling Hospital have been examined, but the table is repeated here for completeness. As has been noted, this is really a special series of children fed exclusively on cow's milk, giving an excessively high incidence of bovine infection. They should be so considered, and general deductions cannot be drawn from them and applied to infant mortality at large.

CASES—FOUNDLING HOSPITAL

Diagnosis	Children Under 6 yrs.		Notes
	H.	B.	
Abdominal Tuberculosis	..	1	
Generalized Tuberculosis	3	3	Two of the bovine cases died directly of exanthemata; the tuberculosis was not altogether negligible. Those two cases not included in the total fatal cases.
Generalized Tuberculosis, including the meninges	1	1	
Totals	4	5	Total cases, 9.

If we consider the Babies' Hospital cases alone the mortality due to bovine infection is six and one-third per cent. The total mortality cannot be considered normal while the Foundling Hospital cases are included. Exclusive of these the general mortality would be a small fraction under 10 per cent. These percentages are believed to give a truthful picture of the percentage limits of mortality and are applicable to the general population of New York City and on the whole to most American cities. The children received at the Babies' Hospital represent the average New York City child, while those at the Foundling Hospital represent the portion fed on cow's milk.

AGE INCIDENCE OF TOTAL FATAL CASES UNDER FIVE YEARS

Diagnosis	Between 4 and 5 Years		Between 3 and 4 Years		Between 2 and 3 Years		Between 1 and 2 Years		Under 1 Year	
	H.	B.	H.	B.	H.	B.	H.	B.	H.	B.
Pulmonary Tuberculosis	..	..	1	..	..	..	2	..	4	..
Abdominal Tuberculosis	..	..	..	..	..	..	..	*2	..	..
Generalized Tuberculosis	1	..	..	1	3	..	5	2	10	1
Generalized Tuberculosis, including Meninges	..	..	1	..	2	..	8	†2	14	..
Tubercular Meningitis	..	..	2	..	5	1	10	1	9	..
Totals	1	..	4	1	10	1	25	7	37	1

*One case bovine type, definite age not given, infant. Not included.
†Case of double infection included here.

If we further correct the application of the percentages deduced, it is evident from the above table that we should apply them mainly to the first three years of life. The cases examined between three and five years are few in number. Further, the pulmonary type of disease becomes more evident at this age and if more of this type of disease had happened to fall in this age period the percentage would have been reduced. The source of our material did not include many cases about this age and only four cases came to us between five and sixteen years, not including the pulmonary cases, the outcome of which we could not determine. We are inclined to believe that around the ages of four to five years the incidence of fatal bovine infection rapidly falls and that one factor in this fall is the increase in the pulmonary types of infection.

Since the completion of Volume V a complete report has been published by Burckhardt of his investigation of surgical tuberculosis. The following tabulation gives the results of this important work. As will be noted, the percentage of bovine infection is higher than one would expect.

Diagnosis of Cases Examined	Adults 16 yrs. and Over		Children 5 to 16 yrs.		Children Under 5 yrs.		Notes
	H.	B.	H.	B.	H.	B.	
Tuberculous Adenitis, Cervical...	5	..	3	1	..	..	
Abdominal Tuberculosis.....	3	..	1	..	..	1	One case, originally classified here, changed to next heading.
Generalized Tuberculosis.....	1	..	..	..	..	..	Clinically the abdominal symptoms only were marked.
Tuberculosis of Bones and Joints	9	1	12	2	4	..	One case (child) age not stated, therefore not tabulated, gave human type.
Genito-Urinary Tuberculosis....	3	..	1	..	..	..	
Tuberculosis of Skin.....	1	..	..	..	..	..	
Totals.....	22	1	17	3	4	1	(1) Total cases, 49.

The observations of Burckhardt on the comparison of the pathological and clinical details in his bovine and human infections in joints are interesting. Of twelve cases of tuberculosis of the knee two were noteworthy by the absence of marked bone lesions. One of them had lasted one year; this was of the human type. The other, which showed a very small bone focus in the tibia only after careful search, had lasted for 13 years. This was of bovine origin. The age of the patient was 19 years. Recovery in both instances was complete.

Of the three cases of tuberculosis of the hip there was one bovine infection. Here again the history was of long duration, *viz.*, eight years. This case (11 years old) also showed only superficial granulations. The two other cases, which were human infections, showed deep bone involvement. The operative results in the bovine case were very good. Another bovine infection of the elbow joint showed only granulations on the capsule. This case, however, had lasted only one year.

Because of these peculiarities in the bovine cases, Burckhardt thinks it would be well to investigate those joint cases showing practically no bone involvement, and especially those cases giving a long history of infection. From the cases already cited one might expect many, if not the majority, of such cases to be bovine infections. This is only a suggestion,

* On the appearance of the preliminary report we had written to Dr. Burckhard and he kindly sent us details of his cases prior to his final publication which would have given us the opportunity of including them in Vol. V had the letter reached us. Due to some clerical error the letter was filed away and not brought to our notice till too late.

and only further cases could give the necessary evidence to prove this possibility.

It is of interest to note that the only distinct bone infection without joint complication due to the bovine bacillus is one reported by Ochlercker. This case gave a long history, namely, 6¾ years. The disease was limited to one metacarpal bone.

Another report is that of Rothe, who publishes a continuation and amplification of Gaffky's work (see Vol. V, p. 154). The bronchial and mesenteric nodes of 100 children up to 5 years of age were examined. The material was from successive autopsies on children dying from any cause. Guinea pigs were inoculated, and if the inoculation were positive cultures were isolated and tested. The results of his work and Gaffky's are given in the following table, copied with slight change from Rothe's article:

Author	Number of Cases and Age	Number Positive	Tuberculosis Present in Both Nodes	Mesenteric Nodes Only	Bronchial Nodes Only	Human Infections	Bovine Infections
Gaffky...	300 under 14 yrs..	57 = 19%	29	11	17	55	(2?) see next paragraph
Rothe...	100 under 6 yrs...	21 = 21%	13	3	5	20	1
Totals.	400.....	78 = 19.5%	42	14	22	75	3 (?)

In the two cases in which Gaffky failed to isolate cultures the great difficulty encountered is good presumptive evidence of their being bovine. They cannot be excluded, and if the figures are used at all they should be considered as bovine, failing proof to the contrary. If these cases are not included, Gaffky's figure should be completely excluded from any consideration of the incidence of bovine infections. Rothe says, "Lässt man die beiden Fälle der 1. Untersuchungsreihe, in denen die Gewinnung einer Reinkultur nicht gelungen war, als unentschieden ausser Rechnung, so verbleiben 76 tuberkulöse Fälle, darunter; 75:98.68 pro cent mit humaner und 1:1.32 pro cent mit bovine infection." What he should have said was, excluding Gaffky's cases altogether, because they are incomplete and inconclusive, that there are left 21 cases of tuberculosis, of which 20 or 95.24 per cent. are human and 1 or 4.76 per cent. are bovine. If Gaffky's cases are considered and the two doubtful cases are classed as bovine, the percentage is very much the same. The following tabulation gives the results of Rothe's work. It is difficult to tabulate his cases, as the details of the post-mortem examinations are not given.

Diagnosis of Cases	Children Under 5 Yrs.			Notes
	Human		Bovine	
Pulmonary Tuberculosis	5	3	1	The bovine case had swollen mesenteric nodes which were considered non-tuberculous on microscopic examination. One of four inoculated guinea pigs became tuberculous.
Abdominal Tuberculosis	..	2	..	Slight latent tuberculosis of mesenteric nodes.
Generalized Tuberculosis	6	3	..	Three cases were latent or slight tuberculosis of lymph nodes.
Tubercular Meningitis	1	..	..	
Totals	20		1	

The above table brings out very strongly the difficulty encountered in tabulating cases of different authors who have had different aims in view. Thus, in one instance slight tuberculosis of the lymph nodes in the thoracic and abdominal cavities is placed under generalized tuberculosis to bring out the fact that the bacilli have disseminated. On the other hand, a more marked tuberculosis involving the lungs with what has theoretically been assumed to be secondary involvement of the intestines and possibly the mesenteric nodes, is classified under pulmonary tuberculosis. Although this seems contradictory, we have attempted to keep certain distinct types of disease separate, but where such types do exist to classify on the basis of the amount of dissemination. In this case (see Vol. V., pp. 134-6) the degree of involvement is noted by placing the slight infections to the right of a vertical line subdividing the space for these cases.

One fact in Rothe's article is very valuable for comparison of the value of different media. Gaffky failed to isolate two viruses, as has been noted. Rothe states that isolation was successful from the first guinea pig passage 17 times, from the second guinea pig passage 10 times, from the third 4 times, and in one case each only after the fifth, eighth and ninth passage. The last two were the bovine strains from the case of pulmonary tuberculosis. He used 2 per cent. glycerine beef serum for isolation. This compares very badly with results obtained with egg media. Except for unavoidable variations in media and in individual technic, the positive results from the first pig, if the pigs are sufficiently tuberculous to give the necessary material, should be 95 per cent. at the very lowest. This includes human and bovine viruses. As a matter of fact, we have found it to be very exceptional to fail even with bovine viruses.

Finally the results of Möllers are to be added. He reports the study of cultures isolated from the sputa of 51 cases of pulmonary tuberculosis. In 10 cases 3 isolations were made at different times, in 9 cases cultures were isolated twice, in the remaining 32 one culture only was isolated. Fifty of these cases were adults; one was an infant.

Bulloch also reports 23 cases of pulmonary tuberculosis yielding cultures of the human type. Adding the preceding reported cases and those of Fabyan (see footnote Vol. V, p. 160), gives the following tabulation:

* The age groups were kindly sent us by Dr. Möllers.

TOTAL SUMMARY OF CASES REPORTED

Diagnosis of Cases Examined	Adults 16 yrs. and Over		Children 5 to 16 yrs.		Children Under 5 yrs.	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary Tuberculosis	363	1?*	3	..	13 | 3	1 | ..
Tuberculous Adenitis, Axillary	1	..	..	..	2	..
Tuberculous Adenitis, Cervical	18	1	17	13	9	8
Abdominal Tuberculosis	13	4	1 | 6	3 | 3	3 | 5	6 | 4
Generalized Tuberculosis, Alimentary Origin	6	1	2 | ..	3 | ..	12 | ..	10 | ..
Generalized Tuberculosis	27	..	3 | ..	1 | ..	20 | 5	1 | ..
Generalized Tuberculosis, including Meninges, Alimentary Origin	..	..	1	..	3	8
Generalized Tuberculosis, including Meninges	4	..	7	..	27	..
Tubercular Meningitis	..	..	1	..	1	2
Tuberculosis of Bones and Joints	26	1	28	3	19	..
Genito-Urinary Tuberculosis	11	..	1	..	..	..
Tuberculosis of Skin	2	..	1	..	1	..
Miscellaneous Cases:						
Tuberculosis of Tonsils	..	..	..	1	..	..
Tuberculosis of Mouth and Cervical Nodes	..	1	..	..	..	..
Tuberculous Sinus or Abscess	1	..	..	..	..	..
Sepsis. Latent Bacilli	..	..	..	..	1	..
Totals	472	9	71	27	124	40

Mixed or double infections, 3 cases.

Generalized Tuberculosis, Alimentary Origin, 30 yrs, Human and Bovine type in mesenteric nodes, Human type in bronchial node.

Generalized Tuberculosis, Alimentary Origin, 5½ yrs. Human type in spleen. Bovine type in mesenteric node.

Generalized Tuberculosis including Meninges. Alimentary Origin. 4 yrs. Human type in meninges and bronchial nodes. Bovine type in mesenteric nodes. Total cases, 746.

*See Addenda for cases of Royal Commission not included here, due to lack of complete details.

COMBINED TABULATION CASES REPORTED AND OWN SERIES OF CASES

Diagnosis	Adults 16 Yrs. and Over		Children 5 to 16 Yrs.		Children Under 5 Yrs.	
	H.	B.	H.	B.	H.	B.
Pulmonary Tuberculosis	644	1?	11	..	23	1
Tuberculous Adenitis, Axillary or Inguinal	2	..	4	..	2	..
Tuberculous Adenitis, Cervical	27	1	36	21	15	21
Abdominal Tuberculosis	14	4	8	7	9	13
Generalized Tuberculosis, Alimentary Origin	6	1	2	3	13	12
Generalized Tuberculosis	29	..	4	1	43	5
Generalized Tuberculosis, including Meninges. Alimentary Origin	..	..	1	..	3	8
Generalized Tuberculosis, including Meninges	5	..	7	..	52	1
Tubercular Meningitis	1	..	3	..	27	4
Tuberculosis of Bones and Joints.	27	1	38	3	26	..
Genito-Urinary Tuberculosis	17	1	2	..	..	..
Tuberculosis of Skin	3	..	1	..	1	..
Miscellaneous Cases:						
Tuberculosis of Tonsils	..	..	..	1	..	..
Tuberculosis of Mouth and Cervical Nodes	..	1	..	..	..	..
Tuberculous Sinus or Abscess.	2	..	..	..	..	..
Sepsis. Latent Bacilli	..	..	..	..	1	..
Totals	777	10	117	36	215	65

Mixed or double infections, 4 cases. Total Cases, 1,224.

Taking the cases given in the total tabulation and combining the important diagnosis under one heading gives us the following table, which shows clearly the percentage incidence of bovine infection. Caution is necessary in applying these figures. They tell nothing but the incidence; the seriousness of the infection is indicated in the preceding tables.

PERCENTAGE INCIDENCE OF BOVINE INFECTION*

Diagnosis	Adults 16 Yrs. and Over	Children 5 to 16 Yrs.	Children Under 5 Yrs.
Pulmonary Tuberculosis	0% †	0%	4.1%
Tuberculous Adenitis, Cervical	3.6%	36%	58%
Abdominal Tuberculosis	22%	46%	59%
Generalized Tuberculosis	2.7%	40%	23%
Tubercular Meningitis (with or without generalized lesions)	0%	0%	13.6%
Tuberculosis of Bones and Joints	3.5%	7.3%	0%

*Exclusive of cases of double infections. In considering pulmonary cases it must, however, be remembered that bovine tubercle bacilli have been isolated from the lung, in cases of generalized tuberculosis in children.

The number of cases under some of the headings is too small to deduce percentages. Reference to the preceding table makes this evident.

† If the two bovine cases of the Royal Commission (see Addenda) were included, the percentage would be 0.3%. We have not included these two cases as the additional human cases could not be included. If we combine the pulmonary cases regardless of age we can then add these cases, giving us a total of 710, exclusive of the one doubtful case. Of these, 3 or 0.42% were bovine infections.

This table gives only the incidence of infection and nothing as to the severity of the disease. This is seen by referring to the main tables, in which we divide the cases according to severity. Under certain diagnoses a great many latent or slight infections are included, which may never have had any effect on the health of the child, had not some intercurrent infection led to death. Furthermore, due to selection of material the number of cases of generalized tuberculosis of alimentary origin is markedly out of proportion and bears no relation to the incidence of these cases in proportion to other types of disease. If we rearrange the figures under these headings, leaving out all but severe types of disease, and consider the selected cases of alimentary tuberculosis separately, the following table gives the results. Only cases under 16 years are considered, as we have only noted the severity of disease in these cases in the tables. The percentage of our cases are given for comparison.

PERCENTAGE OF BOVINE INFECTION (REVISED).

Diagnosis	Children 5 to 16 yrs.		Children Under 5 yrs.	
	Combined Figures	Own Figures	Combined Figures	Own Figures
Abdominal Tuberculosis	66%	50%	69%	75%
Generalized Tuberculosis. Alimentary Origin	60%	....	48%	66%
Generalized Tuberculosis	20%	....	11%	18%
Tubercular Meningitis, secondary to tuberculosis of alimentary type	....	....	72%	....
Tubercular Meningitis (other than preceding)	....	....	6%	5½%

Revising the percentages in this way gives close agreement. The percentages are highest in relatively less common types of tuberculosis. In the two types of tuberculosis which mainly constitute the fatalities in young children the percentages range from 5½ per cent. to 18 per cent. All things considered, we feel safe in saying that the percentage of deaths from bovine tuberculosis in young children, *viz.*, 6⅓ per cent. to 10 per cent., as deduced from our unselected cases in New York City, is applicable to most cities throughout the world whose milk supply is similar to ours.

Addenda.—Since the completion of this final summary of our work the "Final Report of the Royal Commission appointed to inquire into the relations of human and animal tuberculosis" has appeared. Only Part I, *viz.*, the Report, has reached us. The Appendices, which will contain the experimental data and details of the cases examined, are not available. Failing these details, it is impossible for us to include their added cases in our tabulations, nor is it possible to discuss their final conclusions. For this reason we add simply a short résumé of the conclusions given in the report.

Concerning the existence of different types of tubercle bacilli they say: "For purposes of description it is advantageous to distinguish three types of tubercle bacilli, recognizable by their individual characters. These are the human, bovine and avian types. The human type, although so named, is not the only one found in cases of tuberculosis in man. It is the organism present in the majority of such cases, but in some cases of human disease the bacilli present are of the bovine type, and in others the bacilli have special characters distinguishing them from each of the three principal types. In natural cases of tuberculosis in cattle the only type of bacillus present is the bovine type." The conclusions on the differential characters of the human and bovine type are practically identical with our own. All their viruses from man are tabulated as bovine or human in all their characteristics, with the exception of the viruses from cases of lupus. The following gives a short summary of their final results as they tabulate them.

CASES OF HUMAN TUBERCULOSIS OTHER THAN LUPUS.
108 CASES.

Nature of Case	Bovine	Human	Mixed Viruses—Human and Bovine
Primary Pulmonary Tuberculosis	0	14	0
Sputum from Individual Cases of Pulmonary Tuberculosis	2	26	0
General Tuberculosis	0	3	0
Tuberculous Meningitis	0	3	0
Bronchial Gland Tuberculosis	0	3	2 { H. 13 A. D. / H. 60 W. B.
Cervical Gland Tuberculosis	3	6	0
Primary Abdominal Tuberculosis	14	13	2 { H. 49 T. C. / H. 90 I. P.
Joint and Bone Tuberculosis	0	13	1 H. 16 J. H.
Tuberculosis of Testicle, Kidney or Suprarenal. 1 each	0	3	0

The most astonishing thing is the presence of bovine tubercle bacilli in the sputum of two cases. Both patients were over 16 years of age. In one case the examination was repeated after 76, 117 and 118 days with the same results. In the other case a second specimen collected after 118 days gave the same results. Both cases subsequently died. The cause of death in one was given as phthisis; the other apparently had general tuberculosis with intestinal ulceration. At the time of collection of the specimens they were, clinically, cases of primary pulmonary tuberculosis. No autopsy could be obtained.

The other noteworthy point is the number of mixed viruses. In the preceding report the commission gave the results on certain passage experiments without drawing any final conclusions. The changes found, they now conclude, were due to the presence of a mixture of human and bovine viruses in the original viruses. In the case of some of the viruses, for instance H. 13 A. D., such a conclusion is justified The extraordinary fluctuations in virus H. 49 could also be accounted for in this manner. That it is justified in all cases, however, seems almost beyond the possibility of experimental proof. As soon as material has passed through calves a possible experimental error, *viz.*, "spontaneous" tuberculosis of the calf, is encountered. That this error can be surely avoided in each case seems to us almost impossible. Critical judgment, however, must be withheld till every detail of the experiments is published.

The cultures from cases of lupus form a group by themselves. Twenty cases were examined. Culturally the viruses fell into either the human or bovine type. In virulence, however, only one was typically of the bovine type and two of the human type. The cultures were tested by inoculation into calves, rabbits, monkeys, and guinea pigs. In some or all of these animals the virulence, with the exception of the above three cultures, was less than one would expect with one or the other type as determined culturally. The decrease of virulence from the type with some of the viruses was very marked. In a few of these viruses it was possible to raise the virulence to that of the type. It is of interest to note that the only other aberrant cultures isolated were from two horses, and these, like some of the lupus cultures, were culturally of the bovine type, but of degraded virulence. Passage experiments resulted in an increase of virulence to that of the bovine type.

The aberrant cultures were restricted to those isolated from the cases of lupus and equine tuberculosis already described. From the description they are neither typically bovine nor human in all their characters. The most striking thing is that they should have mostly come from one type of disease in man.

As to passage experiments and other means to secure modification of type they report complete inability to cause any change, the only exceptions being the lupus and equine cultures, where it was possible to enhance the virulence of some cultures by passage experiments.

They concluded: "Thus we are inclined to regard transmutation of the bacillary type as exceedingly difficult, if not impracticable, of accomplishment by laboratory procedure, though in view of certain instances in which we obtained from one and the same human body both types of bacillus we are not prepared to deny that the transmutation of one type into another may occur in nature." The lupus cultures and the cultures from the horse, they believe, must be considered naturally modified human or bovine tubercle bacilli, as the only other alternative is to consider them added fixed types.

For the preceding reason virulence cannot be considered a fixed characteristic, which makes it impossible to regard difference of virulence for the calf and rabbit as sufficient to establish the non-identity of the human and bovine types.

"There would therefore remain only slight cultural differences on which to found the conclusion that the human and bovine types represent two distinct organisms. We prefer to regard these two types as varieties of the same bacillus. . . ."

CONCLUSIONS.—Bovine tuberculosis is practically a negligible factor in adults. It very rarely causes pulmonary tuberculosis or phthisis, which causes the vast majority of deaths from tuberculosis in man and is the type of disease responsible for the spread of the virus from man to man.

In children, however, the bovine type of tubercle bacillus causes a marked percentage of the cases of cervical adenitis leading to operation, temporary disablement, discomfort and disfigurement. It causes a large percentage of the rarer types of alimentary tuberculosis requiring operative interference or causing the death of the child directly or as a contributing cause in other diseases.

In young children it becomes a menace to life and causes from 6⅓ per cent. to 10 per cent. of the total fatalities from this disease.

PULMONARY TUBERCULOSIS—ADULTS

Type of Cases Examined:

Diagnosis.....clinically, no autopsies.
Lung involvement.....slight to severe.
Ages.....16 to 50 years.

Number of Cases Examined.....56

Cultures from sputum.....55
" " pleural fluid... 1

Type of Culture Isolated:

Human type I.....51
" " IIa..... 3
" " IIb..... 2

Virulence:

Non-virulent.....56
Virulent.....0

PULMONARY TUBERCULOSIS—CHILDREN

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
582	Sputum	H. Type 1	Non-virulent	M.S.	11 mos.	M.	4 mos.	Pulmonary Tuberculosis
583	"	"	"	G.L.	6½ "	M.	1 mo.	"
667	"	"	"	I.V.	3 yrs.	F.	5 wks.	"
771	Bronchial Nodes	"	"	C.P.	18 mos.	M.	1 mo.	"
772	"	"	"	W.McA.	7 "	M.	1 "	"

582. History: Breast fed with addition of grocery milk diluted; cough and steady loss of weight for four months: Physical examination: Dullness over upper and middle lobe of right lung, numerous râles throughout both lungs, tubular breathing at right apex. Numerous tubercle bacilli in sputum. Discharged from hospital unimproved.

583. History: Not breast fed. Mother died from pulmonary tuberculosis a few weeks after the death of the child. Physical examination (February 20, 1909): Child emaciated and acutely ill. Lungs—Marked dullness over left lung, less marked over lower lobe. Diminished resonance over right lung. Auscultation showed distant bronchial breathing over left upper lobe, with moist râles. Sputum showed many tubercle bacilli. Died a few weeks after.

667. History: Father and mother well. One child died of pulmonary tuberculosis three months ago. Breast fed for one year, then cow's milk and table food. Measles, three months ago. About March 15, 1909, began to have cough, fever, vomiting and constipation. Physical examination (April 2, 1909): Poorly nourished. Lungs—Left upper lobe markedly dull, with bronchial breathing and coarse râles; many râles over left lower lobe and a few over right lung. Sputum contained many tubercle bacilli. Discharged April 23, 1909, unimproved.

771. History: Acute symptoms of entero-colitis and pulmonary tuberculosis. Anatomical diagnosis: Pulmonary tuberculosis, with cavity formation, pleurisy, tuberculosis of bronchial nodes, tuberculous entero-colitis.

772. History: Otitis media and bronchitis following measles. Anatomical diagnosis: Tuberculous abscess of lung, tuberculous, bronchial and mesenteric nodes.

TUBERCULOUS ADENITIS—ADULTS

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
609	Cervical Node	H. Type I	Non-virulent	H.	37 yrs.	F.	2 yrs.	Tuberculous Adenitis
619	Axillary Node	"	"	C.F.	23 "	F.	9 mos.	"
646	Cervical Node	"	"	H.C.	19 "	M.	8 yrs.	"
648	Axillary Node (primary cervical)	"	"	E.N.	17 "	F.	2 "	"

609. History: Two operations for glandular enlargement in neck within two years. The present nodes have been palpable for three months.

619. History: Family and past history negative. Nine months ago noticed swelling under left arm; fluctuated in size. Physical examination: Nodular, moderately hard swelling 2″ x 2″ between breast and left axilla. Another irregular mass in axilla about size of a walnut. Excision. Pathological examination: Tuberculous adenitis. Discharged two weeks later, cured.

646. History: Cervical nodes have been enlarged for past eight years. One or two nodes have broken down and been incised and pus evacuated; after this swellings subsided except under the symphysis menti. Two months ago nodes in left side of neck began to enlarge. Physical examination: Negative, except for enlarged cervical nodes on left side, extending from ear to mid-line parallel to inferior maxilla, largest the size of a walnut. Excision. Nodes were caseous or completely broken down.

648. History: Sister died of tuberculosis. For past two years the lymph nodes of right side of neck have swollen and disappeared alternately. One year ago noticed a lump in left axilla; was weak and had a bad cough at this time; has increased to size of small orange. Physical examination: Emaciated. Lungs negative. Excision of nodes in left axilla and right cervical region. Two nodes from neck, which are broken down and cheesy; left axilla, number of nodes size of a pea up to a peach, which are broken down and cheesy. Pathological report: Tuberculous adenitis. Discharged one month later, cured.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
656	Cervical Nodes	H. Type I	Non-virulent	M.T.	19 yrs.	F.	1 yr.	Tuberculous Adenitis
681	"	"	"	A.K.	34 "	M.	4 yrs.	"
705	"	"	"	C.P.	43 "	F.	7 "	"
707	"	"	"	C.S.	20 "	M.	1 yr.	"

656. History: Past history negative. Six months ago swelling appeared. One month ago another swelling appeared back of the first and has increased rapidly in size; slight cough for six weeks, when swelling appeared. Physical examination: Negative, except for swollen lymph nodes. Two nodes, size of English walnuts, in submaxillary triangle; also chain of nodes anterior to sternomastoid, and between sternomastoid and trapezius enlarged, the largest being size of a walnut. Excision. Discharged three weeks later, cured.

681. History: Sister had tuberculosis. Four years ago lymph nodes removed from over clavicle and right axilla. One year ago further nodes removed from right side of neck. A few weeks after recovery from the latter operation further nodes began to appear. Physical examination: On the right side of the neck below ear there is a mass the size of a walnut; over the right clavicle another mass is palpable. Excision of cervical nodes both sides. Pathological report: Hyperplastic lymphadenitis. Discharged three weeks later, cured.

705. History: Seven years ago, following an attack of influenza, a small swelling appeared in the neck. Five years later this was excised. Another lump appeared above the clavicle and one back of the sternomastoid muscle. These remained the size of a pea. About four months ago after a severe cold a lump began to grow at angle of left jaw, which has increased to the size of a marble. Physical examination: Otherwise negative. Excision; left anterior cervical nodes, one the size of a hickory nut, was caseous, as were some of the smaller nodes. The left supra-clavicular nodes were small and some were caseous. Pathological report: Tuberculous lymphadenitis. Discharged, cured.

707. History: One year ago the lymph nodes of the left side of neck began to enlarge. Physical examination: Anæmic; otherwise negative, except for enlarged lymph nodes, extending from mastoid to clavicle; a few palpable nodes in the right axilla and both groins. Excision. Ran irregular fever of about two degrees above normal. Discharged, cured.

TUBERCULOUS ADENITIS—CHILDREN

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
535	Cervical Nodes	B. Type I	Virulent	N.R.	4 yrs.	F.	1 yr.	Tuberculous Adenitis
536	"	"	"	T.C.	2½ yrs.	F.	5 mos.	"
590	"	"	"	E.G.	2¼ "	M.	2½ mos.	"
605	"	H. Type I	Non-virulent	M.N.	13 "	F.	2½ yrs.	"
627	Axillary Nodes	"	"	C.C.	12 "	M.	?	"

535. History: About one year ago there appeared a tumor the size of a hazelnut in the right side of the neck. This has steadily increased in size. No other symptoms. Physical examination: Negative, except for enlarged tonsils and a mass of nodes in neck about the size of a golf ball. Excision: Nodes removed and about an ounce of greenish yellow pus evacuted. Discharged, healed; general condition good. One month later an abscess appeared in line of scar, which was incised.

536. History: Shortly after an attack of chicken pox about fifteen months ago a lump was noticed in the left side of the neck, which has fluctuated in size. Physical examination: Negative, except for enlarged subtonsillar and submaxillary nodes. Excision: Nodes removed from anterior triangle as far down as the clavicle, also some from beneath the sternomastoid muscle. Discharged, healed.

590. History: Swelling has been present in the neck for about two and one-half months. An abscess formed, which was incised, forming an open discharging sinus. Physical examination: Anæmic child. In the right anterior triangle is a sinus discharging creamy pus. The surrounding nodes are enlarged and adherent to soft parts and to each other. The posterior chain enlarged. Excision of enlarged nodes. Abscess found beneath sternomastoid muscle. Nodes are caseous.

605. History: One sister has bone and gland tuberculosis. Two and one-half years ago was operated on for swellings in both sides of the neck. The wound on the left side never entirely healed. Other lymph nodes have enlarged in the last three months. Physical examination: Poorly nourished. Lungs—Expansion poor; few râles over the bases. Excision of enlarged nodes from both sides of neck. Those on the left side found broken down and there was pus present. Pathologist's report: Nodes vary in size up to hickory nuts and show large tuberculous foci and caseation. Discharged, wound healed.

627. Tuberculous adenitis. Excision. Discharged, healed. No further details.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
636	Cervical Nodes	B. Type I	Virulent	E.C.	6 yrs.	F.	1 yr.	Tuberculous Adenitis
654	"	"	"	R.W.	2½ yrs.	M.	½ "	"
668	"	H. Type I	Non-virulent	J.P.	14 "	F.	6 yrs.	"
669	"	B. Type I	Virulent	L.F.	3½ "	F.	1 yr.	"
670	Axillary Nodes	H. Type I	Non-virulent	R.A.	6 "	M.	?	"

636. History: For the last year the lymph nodes of the neck have been enlarged. A discharging sinus had been present some time. Physical examination: Well nourished. Left side of neck shows large discrete nodes in anterior and posterior triangle, the largest node being the size of an acorn. There is a sinus in the subparotid region. Excision of enlarged nodes, which show cheesy degeneration. Discharged, wound healed.

654. History: There has been a swelling on both sides of the neck for six to eight months. On operation large masses of nodes were removed. Those on the left side were broken down; those on the right side showed caseous spots.

668. History: Began about six years ago with swelling in the left side of neck. About a year and a half ago a mass of six nodes was removed below tip of mastoid, back of angle of the jaw. The nodes were soft and contained large areas of caseation. The wound healed in about two weeks. Physical examination at present time: Well-developed girl, negative, except for local condition. Excision of nodes in anterior and posterior triangles of left side of neck; they vary in size from a millet seed to the size of an acorn. Some of the nodes are friable, others smooth and homogeneous on section. Microscopically they show increased fibrous tissue, with areas of necrosis. Tubercles are few in number.

669. History: About one year ago after an attack of meningitis a swelling was noticed in the left subparotid region. Physical examination: Negative except for local condition. Excision of mass in left subparotid region lying beneath the sternomastoid muscle. Some of the nodes contain fluid pus. Pathologist's report: Tuberculous adenitis, caseous degeneration. Discharged, wound healed. Two months later wound opened and palpable nodes found in upper angle.

670. History: Swelling noticed in axilla three weeks ago. Physical examination: Negative, except for local lesion. Operation, one-half ounce of thick pus and 10 to 12 large inflamed nodes removed. On section they showed caseous areas. Discharged, healed.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
690	Cervical Nodes	B. Type I	Virulent	A.W.	11½ yrs.	F.	1 yr.	Tuberculous Adenitis
691	"	"	"	K.H.	8 "	F.	4½ mos.	"
694	"	"	"	H.T.	3 "	F.	4 "	"

690. History: Duration one year. Small swelling in right subparotid region, which disappeared and then reappeared and then steadily increased in size. Physical examination: Well-developed child. Few fine râles in left apex posteriorly. The only enlarged nodes are in the right subparotid region, where they form a mass the size of a walnut. Excision: Largest node caseous, smaller ones slightly involved. Discharged, healed. No palpable nodes.

691. History: Family history negative. Four and one-half months ago noticed swelling in neck on left side. Two months ago gland noticed on right side. Physical examination: Lungs negative, cervical nodes enlarged. Excision: Nodes of right subparotid group, size of hazelnut, removed; not caseous; also left subparotid group (10 to 12); one larger than hickory nut with caseous center, other not caseous. Pathologist's report: Tuberculous adenitis. Discharged, cured.

694. History: Swelling has been present in right side of neck for four months. Three weeks ago a sinus opened. Physical examination: Negative, except for enlarged lymph nodes. Excision: Nodes massed together, forming dense clusters, very closely adherent to internal jugular; upper nodes cheesy, lower nodes soft but not cheesy. Moderate amount of periadenitis present. Pathological report: Almost entire tissue taken up with large and small recent tubercles, with caseation at their centers. Discharged, healed. General condition excellent.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
682	Cervical Nodes	H. Type I	Non-virulent	T.L.	12 yrs.	M.	2 yrs. (?)	Tuberculous Adenitis
686	Axillary Nodes (Primary Cervical)	"	"	J.C.	11 "	M.	4 yrs.	"
687	Cervical Nodes	"	"	M.E.	10 "	F.	6 mos.	"
705	"	"	"					

682. History: Eight years ago operated on for abscess in left hip. Two years ago a swelling appeared in right submaxillary region, which has slowly increased in size. Physical examination: Well-developed child; negative, except for presence in anterior and posterior triangles of right side of neck of many discrete, enlarged nodes. In the submental region there is a large rounded mass. Excision: Nodes found caseous, size varying up to a hickory nut. Discharged three weeks later, wound healing.

686. History: Father died of consumption. Four years ago was operated upon for enlarged nodes on both sides of neck and after six weeks was apparently cured. Two years later mass began to grow at outer border of left pectoralis major. Three months later a mass of enlarged caseous nodes was removed from the axilla. The present illness has covered three months, a gradually increasing mass being present in the right axilla. Physical examination: Negative, except for mass size of a hen's egg in the right axilla. Excision. Pathologist's report: Tuberculous adenitis; extensive caseous degeneration. Discharged three months later, healed.

687. History: Swellings in both sides of neck for past six months, which have gradually increased in size. Physical examination: Some dullness over apices of lungs. There is a general enlargement of the cervical lymph nodes, varying in size from a walnut to a pea, the largest nodes being in the submaxillary triangles. Excision: Right side, numerous moderately adherent nodes, studded with caseous spots, but not broken down. Wound healed well. One month later left side enlarged, soft and friable; largest showed caseous areas and was the size of a walnut. Moderate periadenitis. Later an abscess was opened on right side and pus evacuated. Discharged, improved. Several nodes still present in both sides of neck.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
695	Cervical Nodes	H. Type 1	Non-virulent	V.B.	3 yrs.	F.	1 yr.	Tuberculous Adenitis
706	See 687							
712	Cervical Nodes	B. Type 1	Virulent	A.P.	8 "	M.	1 "	"
721	"	H. Type 1	Non-virulent	I.C.	2½ "	F.	?	"
760	"	B. Type 1	Virulent	G.deL.	3 "	M.	6 mos.	"

695. History: For past year a gradually increasing swelling has been present in the right side of the neck below the angle of the jaw. General condition has remained good. Physical examination: Negative, except for enlarged nodes. Excision: A subparotid abscess found and two large caseous nodes. Many smaller nodes along carotid and jugular. Discharged, wound not quite healed. Some cough for a week, but general condition good.

712. History: There has been a swelling below angle of jaw on the right side for the last year. This has gradually increased in size. General health has been good. Physical examination: Negative, except for local condition. Excision: In submaxillary triangle a small abscess present. Tonsillar node size of a pigeon's egg. Variable sized nodes in subparotid region, showing areas of caseation. Considerable periadenitis present. Discharged, healed;; some induration about wound.

721. History: Swelling only noticed for three weeks. Measles four weeks ago. Physical examination: Negative, except for hard swelling at angle of the jaw, right side. Excision: Three large and several small nodes excised; large mass found under the parotid; also enlarged nodes in the deep chain. Large nodes show central caseation. Some pus and surrounding inflammation. Healing was slow. Secondary incision to evacuate pus. Discharged in good condition.

760. History: Lymph nodes of neck excised. Subsequent history favorable; recovery complete; child in good health.

ABDOMINAL TUBERCULOSIS—ADULTS

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
637	Omentum	H. Type 1	Non-virulent	D.T.	22 yrs.	F.	.....	Tubercular Peritonitis

Note.—See generalized tuberculosis, adults, Nos. 308 and 336, for other cultures from abdominal organs.

637. History: No details further than the diagnosis confirmed at operation.

ABDOMINAL TUBERCULOSIS—CHILDREN

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
533	Mesenteric Nodes	B. Type 1	Virulent	T.J.	Infant	M.	?	Tuberculosis of Intestines and Mesenteric Nodes
604	See 155							
708	Cervical Nodes	"	"	A.G.	14 mos.	M.	5 wks.	Tuberculosis of Peritoneum and Cervical Lymph Nodes
709	Peritoneum	"	Virulent					
723	Peritoneum and Fluid	H. Type 1	Non-virulent	P.M.	4½ yrs.	F.	1⅔ yrs.	Tuberculous Peritonitis

533. History: Foundling child. Boarded out under unhygienic surroundings. Fed on milk which was said to have been scalded. Further details not obtainable. Autopsy: Nutrition poor. Rachitic. Cervical lymph nodes not enlarged. Pleura on both sides normal. Lungs—Right, middle lobe solid; no crepitation; on section exudes pus on pressure; gray without distinct markings. In lower portion small abscess containing yellow pus. Left, some congestion and atelectasis posteriorly. Mediastinal lymph nodes normal. Bronchial nodes hyperplastic. Heart normal. Liver enlarged and fatty. Spleen and pancreas normal. Kidneys negative. Small intestine negative. Large intestine, numerous large tuberculous ulcerations from cæcum to anus. Cæcum itself is one large abscess cavity, the whole interior being deeply ulcerated. Mesenteric lymph nodes very large and caseous. The lung condition in this case was considered pneumonic, but no microscopic examination for the presence of tuberculosis was made.

708. History: Family history negative. Breast fed till five months old, with some bottle feeding; no breast feeding since. Has been ill for five weeks; irritable; losing weight. Abdomen has gradually increased in size; occasional vomiting. Physical examination, April 23d: Abdomen distended; lymph nodes below left jaw size of an almond; otherwise negative. On May 29th rolled up mass found in abdomen. Operation June 1st: Peritoneum, many adhesions, and studded with tubercles of varying size, mostly soft and necrotic. Child died June 17th. No autopsy.

723. History: Peritonitis following measles. Abdomen opened twice. Tubercles found thickly scattered over the peritoneum.

GENERALIZED TUBERCULOSIS—ADULTS

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
761	Axillary Lymph Node	H. Type	Not Tested	R.R.	33 yrs.	M.	?	Generalized Tuberculosis including Meninges

Post-mortem.—Lymph node removed and cerebro-spinal fluid aspirated. The latter contained tubercle bacilli.

GENERALIZED TUBERCULOSIS—CHILDREN

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
523	Cerebro-spinal Fluid	H. Type 1	Non-virulent	W.U.	1 yr.	M.	2 wks.	Generalized Tuberculosis including Meninges
524	"	"	"	.....	3½ yrs.	..	4 "	"

523. History: Breast fed. Illness began 10 days ago with projectile vomiting. No rigidity or convulsions. Has been restless for past month. Died January 10, 1909. Autopsy. Anatomical diagnosis: Tuberculosis of lungs, bronchial lymph nodes, liver, spleen and intestines.

524. History: Family and previous history negative. For three weeks child had been running a temperature from 101° to 104°. No vomiting or headache and no cough till last few days. Physical examination negative, except for some enlargement of liver and spleen. The child did poorly; temperature remained high and developed some sub-crepitant râles. The leucocyte count was only 7,600, with 65 per cent. of polynuclears. Two c. c. of blood was collected in citrate solution and few acid-fast bacilli found. At the same time 30 c. c. of clear cerebro-spinal fluid were obtained which contained tubercle bacilli. No clinical evidence was present of meningitis. The child died a few days later, the final clinical diagnosis being acute miliary tuberculosis. No autopsy.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
531	Bronchial Nodes	H. Type 1	Non-virulent	V.T.	8 mos.	F.	1¼ mo.	Generalized Tuberculosis including Meninges
532	Mesenteric Nodes	"	"					
559	Cerebro-spinal Fluid	"	"	F.C.	1 yr.	M.	2 wks.	"
560	Bronchial Nodes	"	"					
607	Cerebro-spinal Fluid	"	"	F.B.	10 mos.	F.	3 mos.	"
616	Mesenteric Nodes	"	"					

531. History: Mother tuberculous for fifteen months. Child has been with her up till three months ago. Breast fed, then Eskay's food. Pneumonia four weeks ago. Since then she has not been well. Two weeks ago child had convulsions. Has been febrile since attack of pneumonia. Physical examination (January 16, 1909): Well nourished, apathetic; respirations labored; fontanels bulging

and tense; knee jerks exaggerated. Kernig's sign and opisthotonos present at times. Lumbar puncture gave clear fluid, in which tubercle bacilli were found. Rigidity and apathy continued, convulsions supervened and child died January 20, 1909. Autopsy. Anatomical diagnosis: Miliary tuberculosis of pleura, lungs, spleen, lymph nodes, liver, spleen and intestines, pulmonary cavity, cheesy and simple broncho-pneumonia.

559. History: No tuberculosis in family. Breast fed till present time, with addition of some cow's milk for last five months. Child seemed well till few days before admission, except for slight cough and fever which was present for two weeks. Had convulsions on day of admission (January 28, 1909). Physical examination: Well nourished, but acutely ill. Eyes—Pupils contracted; react sluggishly. Slight opisthotonos. No Kernig's sign. Child comatose. Lumbar puncture gave 70 c. c. clear fluid. Signs of involvement of lungs. Symptoms became aggravated and child died February 4, 1909.

607. History: Father possibly tuberculous. Breast fed six months, then condensed milk. Attack of bronchitis in January, 1909. Cough has persisted till present. Some fever at night; has lost weight. February 22d, vomiting attacks, projectile in character. March 4, became drowsy; stiffness and convulsions followed. Physical examination (March 16, 1909): Pale, comatose; bulging fontanels; pupils do not react; slight rigidity of neck; no Kernig's sign. Lumbar puncture, 35 c. c. clear fluid containing tubercle bacilli. Coma deepened, general convulsions supervened and death occurred March 16, 1909. Autopsy. Anatomical diagnosis: Acute general miliary tuberculosis, involving lungs, pleura, peritoneum, spleen, liver, pia mater, intestines, cheesy pneumonia, broncho-pneumonia, tuberculous meningitis, cheesy tubercles of lymph nodes.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
608	Sputum	H. Type I	Non-virulent	A.G.	8 mos.	F.	1 mo.	Generalized Tuberculosis
615	Mesenteric Nodes	"	"	C.M.	15 "	M.	5 mos.	"
616	See 607							
617	Cerebrospinal Fluid	"	"					
633	"	"	Not Tested					Generalized Tuberculosis
643	Bronchial Node	"	Non-virulent	J.D.	7½ "	M.	18 days ?	including Meninges
644	Mesenteric Node	"	"					

608. History: No tuberculosis in family. Breast fed since birth. Had cough and fever for two weeks, which subsided. On February 22d cough and fever began again. Physical examination (March 5th): Poorly nourished; dullness over entire left lung; breath sounds absent over lower node; diffuse râles over remainder of both lungs. Child improved somewhat and was discharged March 11. On March 15 child collapsed and was taken to another hospital, where

it died the same day. Autopsy. Anatomical diagnosis: Pyopneumothorax, acute broncho-pneumonia, tuberculosis, tubercles in peritoneum, spleen, liver, kidneys, general caseous tuberculous adenitis, tuberculous ulcers of large and small intestines.

615. History: Family history negative. Always well till five months ago, when he began to lose weight. In February had enlargement on left side of neck, which was opened. Had measles in January. Symptoms have been vomiting, diarrhœa, cough and weakness. Physical examination (March 8, 1909): Rachitic, emaciated child. Signs of involvement of lungs. Liver and spleen enlarged. Died March 14, 1909. Autopsy. Lungs, not collapsed, many pleural adhesions. Lower right lobe shows a cavity filled with caseous material. All mediastinal and bronchial nodes show numerous tubercles. Diaphragm shows large masses of tubercles at junction with ribs. Peritoneum, many adhesions, covered everywhere with tubercles. Mesenteric nodes enlarged and contain tubercles. Spleen much enlarged; shows numerous tubercles.

617. History: One child died of tubercular meningitis in January. Mother's brother, who lived with family and cared for children, has pulmonary tuberculosis. Breast fed till present. Onset March 10; projectile vomiting; became irritable, then drowsy. Physical examination: Well nourished; drowsy; fontanel bulging; pupils react slowly; marked strabismus. Lumbar puncture gave 60 c. c. of clear fluid containing tubercle bacilli. Symptoms became worse; child stuporous. Died March 28th. Autopsy. Anatomical diagnosis: Broncho-pneumonia, cheesy pneumonia (cavity), miliary tuberculosis of lungs, pleura, lymph nodes, spleen, liver, pericardium, peritoneum and intestines.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
634	Cerebro-spinal Fluid	B. Type I	Virulent	M.H.	13 mos.	F.	3 wks. ?	Generalized Tuberculosis including Meninges
651	Mesenteric Nodes	H. Type I	Non-virulent					
640	Cerebro-spinal Fluid	"	"	A.S.	1¾ yrs.	M.	?	"
641	Mesenteric Node	"	"					
642	Bronchial Node	"	"					
643 and 644	See 617							

634. History: No tuberculosis in family. Breast fed with addition of cow's milk since two months old. Illness started March 12th with vomiting, diarrhœa, fever, fretfulness and slight stupor. Physical examination: Poorly nourished; drowsy; fontanel bulging; no eye symptoms; knee jerks increased and ankle clonus present. No signs in lungs. Stupor gradually increased, severe convulsions occurred and child died April 1st. Autopsy. Anatomical diagnosis: Broncho-pneumonia, simple and tuberculous, acute miliary tuber-

culosis of lungs, liver, spleen, kidneys, tuberculous meningitis and adenitis. Lungs—No pleurisy; left, upper lobe well areated with few recent miliary tubercles; lower lobe, scattered areas of broncho-pneumonia in posterior border; one-half inch above the base there is a cheesy triangular area three-quarter inch in diameter, surrounding a bronchus. Number of tubercles throughout rest of lung. Right upper and middle lobes show a superficial strip of broncho-pneumonia. A few small tubercles scattered throughout the lung. Bronchial lymph nodes on both sides enlarged and cheesy. Heart negative. Spleen not enlarged; scattered early tubercles. Liver fatty and studded with recent tubercles. Peritoneum and intestines show no tuberculous lesions. Mesenteric nodes scarcely enlarged. Kidneys show few recent tubercles. Brain—Exudate and tubercles over areas of from one-quarter to one-half inch near the inner border of left hemisphere, at the right ascending parietal convolution a corresponding area. There is a mass of closely studded tubercles at the upper border of the second left occipital convolution. A few scattered tubercles over rest of cortex. Pia mater everywhere is cloudy and beneath it is a small amount of yellowish exudate in all the sulci. There is a layer of exudate over the entire interpeduncular space, pons and medulla, extending down into the cord. No tubercles or exudates on the cerebellum. The lower surfaces of the occipital lobes show some tubercles. The ventricles are not distended, but show tubercles on floor of anterior horns.

640. History: No tuberculosis in family. Breast fed thirteen months; soft diet since. Pertussis in January. Onset of present illness March 24th; fretfulness and fever. Convulsion, stupor and strabismus followed. Physical examination (March 27th): Apparently dying; rigidity and opisthotonos marked. Stupor deepened and child died March 28th. Autopsy. Anatomical diagnosis: Acute fibrinous pleurisy, broncho-pneumonia, and atelectasis, acute miliary tuberculosis of liver, lungs, spleen and lymph nodes.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
650	Mesen-Node	B. Type I	Virulent	H.L.	22 mos.	M.	4 mos.	Generalized Tuberculosis
651	See 634							
660	Cerebro-spinal Fluid	H. Type I	Non-virulent	A.P.	8½ "	F.	5 " ?	Generalized Tuberculosis including Meninges

660. History: Breast fed, then condensed milk. Symptoms began five weeks ago with cough, swelling in right side of neck and reddened throat. Gained weight till three months old; then steadily lost. Three days ago child became restless, then drowsy, with muscular twitching, followed by convulsion and rigidity. Physical examination (April 12th): Poorly nourished. Pupils unequal; opisthotonos marked; child semi-comatose. Lumbar puncture gave 60 c. c. of clear fluid; contains numerous tubercle bacilli. Child became comatose and died April 15th. Autopsy. Anatomical diagnosis: Tuberculous pleurisy, localized tuberculous peritonitis, acute miliary tuberculosis of lungs, liver, spleen, kidneys, intestines, myocardium, tuberculous lymph adenitis.

650. History: No tuberculosis in family. Breast fed one month, then cow's milk unheated. Later discovered that woman boarder had tuberculosis of lungs. Had swelling in neck in December, 1908, which was operated on. Has coughed for two months; low fever, night sweats, and loss of weight. Physical examination (March 13, 1909): Ill-nourished. Left cervical node enlarged and discharging; marked signs of involvement of lungs; dactylitis of great toe. Tubercle bacilli present in sputum. Scars on skin which were considered tuberculides. Child continued febrile and gradually grew weaker. Died April 1, 1909. Autopsy. Anatomical diagnosis: Osteo-myelitis of first metatarsal bone of left foot, tuberculous pleurisy, atelectasis, tuberculous broncho-pneumonia with cavities, miliary tuberculosis of lungs, liver, spleen, kidney, lymph nodes and intestines, tuberculous perisplenitis, fatty liver, oldest lesion in left lung. Body emaciated. Left big toe, pocket of cheesy material involving tendon and periosteum of first metatarsal bone. Head of first phalanx is roughened, joint not involved. Left cervical nodes enlarged; incision over them. Skin shows round, dry, desquamating lesion on abdomen, knee and back. Lungs —No pleurisy. Right, scattered throughout are miliary tubercles. Upper and middle lobes well aerated. Lower lobe contains area of atelectasis, surrounded by areas of broncho-pneumonia. Left, adherent to chest wall, diaphragm and pericardium. In these adhesions are cheesy areas averaging one inch in diameter and one-quarter inch in thickness. These are scattered all over lung. There is a cavity at the upper border of the lower lobe, a smaller one just above the lingula. The apex contains a cavity the size of a large walnut. The lung substance is almost solid and studded with cheesy tubercles and cheesy masses. Bronchial nodes enlarged, more on left side; all contain cheesy tubercles. All the mediastinal nodes are enlarged. Spleen not enlarged, but is adherent to the diaphragm by adhesions, containing cheesy masses, and is studded with gray tubercles. Kidneys, two to three tubercles on surface. Mesenteric nodes slightly enlarged, but nearly all contain cheesy tubercles. One ulcer in upper part of jejunum.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
672	Cerebro-spinal Fluid	H. Type 1	Non-virulent	T.G.	6½ mos.	M.	2 mos.	Generalized Tuberculosis including Meninges
679	Bronchial Node	"	"					
688	Lymph Node (Source?)	"	"	E.O'R.	7½ "	F.	?	"
689	Cerebro-spinal Fluid	"	"	L.B.	8 "	F.	?	"
692	Bronchial Nodes	"	"					

672. History: No tuberculosis in family. Breast fed, supplemented with condensed milk. Has not been well since February. Cervical nodes behind ear enlarged for two months. Physical examination (April 7th) showed no nervous symptoms. Some signs in lungs. April 16th, began to be drowsy. Symptoms then became marked

and child died April 24th. Autopsy. Anatomical diagnosis: Broncho-pneumonia, pulmonary œdema, cavity formation, miliary tuberculosis, liver, spleen, kidneys, lymph nodes and peritoneum.

688. History: No tuberculosis in family. Breast fed one week, then cow's milk; has never gained in weight, constant loss. On April 7th started to cough and had fever. Physical examination (April 13th): Acutely ill; badly nourished; slight facial palsy on right side. Signs in lungs. Nervous symptoms never marked. Child died May 3. Autopsy. Anatomical diagnosis: Tubercular meningitis, cheesy pneumonia, miliary tuberculosis of lungs, liver, spleen, cheesy tuberculosis of lymph nodes, tuberculous ulcers of intestines.

689. History: No tuberculosis in family. Breast fed for two months, later cow's milk. Child boarded out for a while. Onset April 26th with cough and fever; very drowsy; convulsions and rigidity followed. Physical examination (May 3): Poorly nourished; fontanels depressed; eyes turned to left; pupils react slowly. Marked signs in lungs. Child is semi-comatose; has slight opisthotonos and some rigidity. Lumbar puncture gave 35 c. c. of clear fluid, with many tubercle bacilli; became comatose, showed clonic convulsions and died May 6th. Autopsy. Anatomical diagnosis: Acute miliary tuberculosis of liver, spleen, glands, intestines, lungs and right kidney, acute pulmonary tuberculosis and broncho-pneumonia.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
698	Mesenteric Nodes	H. Type 1	Non-virulent					
699	Bronchial Nodes	"	"	M.C.	2 yrs.	F.	?	Generalized Tuberculosis including Meninges
700	Cerebro-spinal Fluid	"	"					
713	Tracheal Node	"	"	P.M.	19 mos.	M.	?	"
714	Mesenteric Node	H. Type*	"					
717	Spleen	B. Type	Virulent	H.K.	$1\frac{5}{12}$ yrs.	M.	?	"
718	Meninges	"	"					

*Lost by contamination, full details of type not determined.

698. History: Family history negative. Breast fed eight months, then milk from Straus laboratory; later table food. Measles in February. On March 3d child seemed to have pain in ear; very irritable. On May 8th drowsiness, which has increased steadily; fretful if disturbed. Physical examination (May 10th): Emaciated; pupils dilated, but react. Otitis of right ear; lungs negative. Child sleeps constantly, roused with great difficulty; slight opisthotonos; no Kernig's sign or rigidity. Lumbar puncture gave 60 c. c. clear fluid. A second puncture gave 65 c. c., in which tubercle bacilli were found. Coma supervened and child died May 16th. Autopsy. Anatomical diagnosis: Acute miliary tuberculosis of lungs, liver, spleen, lymph nodes and kidneys, broncho-pneumonia, cheesy pneumonia. Brain—Examination not permitted.

713. History: Family history negative. Fall one month ago. On admission apathetic facial paralysis and strabismus; moderate opisthotonos. Physical examination: Negative. Lumbar puncture gave 30 c. c. of fluid containing tubercle bacilli. Condition remained unchanged; convulsions, followed by death.

717. Autopsy: Generalized tuberculosis. Foundling child. No other details obtainable.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
748	Bronchial Nodes	H. Type I	Non-virulent	J.M.	8½ mos.	M.	3 wks.	Generalized Tuberculosis
749	Bronchial Node	"	"					
750	Mesenteric Node	"	Not Tested	I.C.	18 mos.	F.	?	"
751	Bronchial Node	"	Non-virulent					
752	Mesenteric Node	"	"	A.B.	2 yrs.	M.	?	"
755	Cerebrospinal Fluid	"	"	L.H.	8 mos.	F.	?	Generalized Tuberculosis including Meninges
756	Bronchial Node	"	"					
764	Mesenteric Node	B. Type	Virulent	A.P.	3 yrs.	F.	?	Generalized Tuberculosis Alimentary Origin
766	Mesenteric Node	H. Type I	Not Tested	M.K.	2$\frac{1}{0}$ "	F.	4 mos.	Generalized Tuberculosis
767	Bronchial Nodes	"	Non-virulent					
768	Mesenteric Nodes	H. Type I	"	P.E.	6½ mos.	M.	?	"

748. History: Clinically disease followed course of a progressive pulmonary tuberculosis. Anatomical diagnosis: Acute miliary tuberculosis of lungs, liver, spleen, kidneys and lymph nodes, tuberculous enteritis, broncho-pneumonia.

749. History: Clinically diagnosed as pulmonary tuberculosis. Anatomical diagnosis: Miliary tuberculosis of lungs, liver, spleen and kidneys, cheesy tuberculous lymph nodes.

751. History: No definite history. Clinical diagnosis: Pulmonary tuberculosis. Anatomical diagnosis: Extensive cheesy tubercles in cerebellum, solitary tubercle in cerebrum, cheesy tuberculosis of lungs and bronchial nodes, broncho-pneumonia and chronic pleurisy, tuberculous entero-colitis.

755. History: Acutely ill eleven days before admission. Tubercle bacilli found in lumbar puncture fluid. Anatomical diagnosis: Acute miliary tuberculosis, broncho-pneumonia, purulent bronchitis, croupous colitis.

764. History: Anatomical diagnosis: Tuberculous enteritis with extensive ulceration involving the entire length of the small intestine, tuberculous lymph adentitis, mesenteric, retro-peritoneal, anterior and posterior mediastinal and cervical. The abdominal nodes formed a large packet, pushing the intestines forward, the individual nodes being completely caseated. Acute general miliary tuberculosis of peritoneum, lungs, liver, spleen and right tonsil. The absence of older lesions in the lungs and the slight involvement of the peribronchial lymph nodes suggested a primary intestinal infection.

766. History: Anatomical diagnosis: Generalized tuberculosis, tuberculous ulcerative colitis.

768. History: Clinically showed cough and fever. Anatomical diagnosis: Tuberculosis of lungs with cavity, tuberculosis of lymph nodes, of liver and spleen, purulent bronchitis, chronic interstitial pneumonia.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
770	Bronchial Nodes	H. Type	Non-virulent	M.N.	10 mos.	F.	?	Generalized Tuberculosis including Meninges
774	Mesenteric Nodes	"	Not Tested	F.de C.	3 "	M.	10 mos.	"
775	Bronchial Nodes	"	"					
776	"	"	"	M.L.	11 "	F.	10 "	"
779	Bronchial Node	"	"	R.H.	1 yr.	F.	?	"
796	"	"	"					
797	Mesenteric Node	"	"	S.H.	$2\frac{10}{12}$ yrs.	M.	8 mos.	"
798	Cerebro-spinal Fluid	"	"					
814	"	"	"	J.S.	7 mos.	M.	?	"

770. History: Acutely ill for thirteen days. Lumbar puncture showed presence of tubercle bacilli. Anatomical diagnosis: Tuberculosis of bronchial nodes, liver, spleen, mesenteric nodes, intestines and lungs. No details given as to brain and cord.

774. History: Weaned at two weeks. Diarrhœa and cough the main symptoms. Mother probably tuberculous. Anatomical diagnosis: General miliary tuberculosis, caseous pneumonia.

766. History: Meningeal symptoms for 12 days. Lumbar puncture positive. Anatomical diagnosis: Tuberculosis of lungs, liver, spleen, bronchial nodes, pancreas and intestines.

779. History: Ill with meningitis for 11 days. Anatomical diagnosis: Miliary tuberculosis of lungs, spleen and kidneys, cheesy pneumonia, cheesy tubercles of lymph nodes, tuberculous ulcers of intestines. Post-mortem lumbar puncture gave fluid containing tubercle bacilli.

796. History: Anatomical diagnosis: Tuberculous meningitis, generalized tuberculosis.

814. History: Meningeal symptoms for 17 days. Tubercle bacilli in fluid from lumbar puncture. Anatomical diagnosis: Miliary tuberculosis of brain, lungs, spleen, liver and kidneys, tuberculous enteritis.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
821	Bronchial Node	H. Type	Not Tested	C.V.	2 mos.	M.	3 wks.	Generalized Tuberculosis involving Meninges
822	Mesenteric Node	"	"					

821. History: Clinically meningitis, confirmed by lumbar puncture. Anatomical diagnosis: Generalized tuberculosis, involving lungs, liver, spleen, kidneys, mesenteric nodes, intestines.

TUBERCULAR MENINGITIS—CHILDREN

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
579	Cerebrospinal Fluid	H. Type 1	Non-virulent	F.McG.	5 mos.	F.	2 wks.	Tubercular Meningitis
580	"	"	"	J.C.	3 yrs.	M.	3 wks.	"
584	"	"	"	W.M.	3 "	M.	14 mos.	Tuberculosis of Hip Joint and Meningitis

579. History: Father has pulmonary tuberculosis. Breast fed till present. About February 8th began to have fever. This was followed by forcible vomiting, stupor and retraction of head. Physical examination (February 18): Well nourished; fontanels bulging and tense; pupils equal; do not react to light; no other physical signs. Lumbar puncture gave 60 c. c. clear fluid, in which tubercle bacilli were found. Convulsions became constant and child died February 22d.

580. History: No tuberculosis in family. Breast fed, then table food. January 27th, showed drowsiness; three days later rolled eyes and twitched. The following day there was paralysis of left side. Physical examination: Stuporous, irritable when disturbed; left facial paralysis; pupils equal and react to light. Lungs—Respiration slow, feeble and irregular; no abnormal physical signs. Spastic paralysis of right arm and leg. No opisthotonos or Kernig's sign. Culture from throat gave diphtheria bacilli. Lumbar puncture gave 60 c. c. clear fluid. No tubercle bacilli found. Child improved somewhat; then became excessively irritable; neck rigid. Discharge from ear gave diphtheria bacilli. A second lumbar puncture gave 110 c. c. of clear fluid and showed many tubercle bacilli. Child became more stuporous, convulsions followed and he died February 19th.

584. History: Family history negative. Entered hospital August 25, 1908, with tuberculous hip disease, which has given symptoms for eight months. On February 19, 1909, patient became restless and had a convulsion, which lasted at intervals with clonic spasms for a couple of hours. Pupils became dilated, spine rigid, Kernig's sign present, child semi-conscious. Lumbar puncture, fluid under tension, containing tubercle bacilli. Child gradually became worse and died February 26, 1909.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
611	Cerebro-spinal Fluid	H. Type I	Non-virulent	C.C.	9½ mos.	F.	?	Tubercular Meningitis
612	"	"	"	J.K.	7 "	M.	?	"
671	"	"	"	C.L.	2½ yrs.	F.	3½ wks.	"
676	"	"	"	C.F.	22 mos.	M.	3 wks.	"

611. History: Another child in hospital for cervical adenitis. Friend of mother's had pulmonary tuberculosis and played with the children. Breast fed for eight months; cow's milk for last two weeks. Child has lost weight for last five months; cough for last three months; slight fever. Physical examination (March 13): Emaciated, cyanotic. Lungs—No dullness; diffuse râles; respiration noisy. March 13th, cyanosis increasing; vomited several times. Marked rigidity, with fontanels bulging; no coma. The presence of meningitis was doubtful. Child died March 13th. A puncture made after death gave fluid containing tubercle bacilli.

612. History: No tuberculosis in family. Breast fed till two weeks ago. Onset February 2d; vomiting, projectile in character; slight cough. On March 6th the child became drowsy; stupor gradually deepened; has had one convulsion. Physical examination (March 10): Well nourished; comatose; fontanel bulging and tense; pupils dilated; do not react to light; internal strabismus. Lungs normal. Knee jerks absent; no Kernig's sign, opisthotonos or rigidity. Lumbar puncture gave 35 c. c. fluid containing tubercle bacilli. Child became comatose. Removed from hospital by parents March 11th.

671. History: No tuberculosis in family. Never nursed. Condensed milk (?) until one year, then table food. On April 5th child showed some weakness. One week later forcible vomiting began, which has continued until present. Drowsiness present for last three days; some twitching and fever. Physical examination (April 19th): Well nourished. Lungs almost flat posteriorly, with faint breath sounds. Vocal resonance normal. Slight opisthotonos and Kernig's sign. Child became more drowsy. Lumbar puncture gave only 10 c. c. of clear fluid, which contained many tubercle bacilli. Sputum negative for tubercle bacilli. Coma supervened with convulsive moments. Temperature rose to 105° and child died April 29th.

676. History: Family history negative. Breast fed for one year, then cow's milk. Symptoms began April 11, fever, drowsiness, one convulsion, later internal strabismus. Physical examination (April 23): Internal strabismus, tremor of hands and marked rigidity present. Pupils are constantly dilating and contracting without reference to light. Later slight convulsions occurred; neck and extremities very rigid. Respiration irregular, finally Cheyne-Stokes type, and child died May 1st.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
677	Cerebro-spinal Fluid	H. Type 1	Non-virulent	E.C.	10 mos.	M.	2 wks.	Tubercular Meningitis
678	"	B. Type 1	Virulent	J.K.	14 "	M.	3 "	"
684	"	H. Type 1	Non-virulent	G.F.	1½ yrs.	M.	3 "	Tuberculosis of Meninges and Lungs
704	"	"	"	P.M.	2 "	F.	4 "	Tubercular Meningitis

677. History: No definite history of tuberculosis in family, but one child died of meningitis two years ago. Breast fed with addition of cow's milk for last four months. Well until April 11th, when child had general convulsion and vomited two or three times a day. Gradually increasing drowsiness until April 22d, when he had two convulsions; since then stupor. Physical examination (April 24): Limp, cyanotic, dying; pupils react to light; no abnormal signs in lungs. Child died same day. Post-mortem lumbar puncture gave 45 c. c. of slightly turbid fluid, containing tubercle bacilli.

678. History: Family history negative. Breast fed; received milk occasionally. Child very fond of butter. April 7th began vomiting two or three times a day; then child became restless. On April 19th stupor noticed; child could not see. There was some twitching, but no convulsions. Physical examination (April 23): Pupils dilated, equal, react to light. Lungs—A few sonorous râles posteriorly at right base. Opisthotonos and Kernig's sign present. The next day convulsions began and were almost continually present for two days. Lumbar puncture gave 30 c. c. of clear fluid containing tubercle bacilli. None found in sputum or fæces. Child comatose and died April 27th.

684. History: Family history negative. Never breast fed, condensed milk for three months, then cow's milk. On April 9th child became fretful; later there was forcible vomiting and fever. Slight cough. Physical examination (April 26): Pupils react slowly. Lungs—Few scattered râles anteriorly and posteriorly. Slight opisthotonos, Kernig's sign present and marked rigidity, convulsive movements of arms and legs. Soon afterward a general convulsion occurred, and lumbar puncture gave 115 c. c. of clear fluid showing tubercle bacilli. Several general convulsions followed and child became comatose. Tubercle bacilli in sputum. Symptoms increased and child died April 28th.

704. History: Family history negative. Breast fed eighteen months, then table food. Present illness began four weeks ago, when child had two convulsions; said to have had broncho-pneumonia at the time. About May 25th opisthotonos and drowsiness appeared. Cough and fever since convulsions. Physical examination (May 26): Emaciated; drowsy; pupils react to light; marked nystagmus, but no strabismus. Lungs normal except for few râles at angle of right scapula, and just above right nipple. Abdomen retracted;

spleen enlarged. In median line below umbilicus few small nodules about size of bean are palpable. Body completely relaxed. Lumbar puncture gave 60 c. c. of clear fluid containing tubercle bacilli. Child died May 31st.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
754	Cerebro-spinal Fluid	H. Type 1	Non-virulent	G.G.	2 yrs.	F.	37 days	Tubercular Meningitis
757	"	"	"	R.E.	15 mos.	F.	7 "	"
769	"	"	"	F.E.	18 "	F.	2 wks.	"
777	"	B. Type	Virulent	W.S.	2 yrs.	M.	40 days	"
789	"	H. Type	Not Tested	H.	11 "	M.	4 "	"
790	"	"	"					
791	"	"	"	J.S.	16 mos.	F.	12 "	"
800	"	"	"	A.C.	22 "	M.	5 wks.	"
805	"	"	"	S.G.	17 yrs.	M.	3 "	"
809	"	"	"	T.	8 mos.	F.	2½ "	"
811	"	"	"	J.D.	2 yrs.	F.	2 "	"
815	"	"	"	R.M.	6 mos.	M.	6 mos.	Tuberculosis of Meninges and Lungs
817	"	"	"	H.H.	20 "	F.	16 days	Tubercular Meningitis
818	"	"	"	C.C.	20 "	F.	1 mo.	Tuberculosis of Lungs and Meninges
819	"	"	"	F.G.	20 "	M.	?	Tubercular Meningitis
820	"	"	"	J.C.	2½ yrs.	M.	1 mo.	"

754. History: Diagnosis on clinical findings. Extent of disease elsewhere not known. No autopsy.
757. History: Diagnosis clinical and by lumbar puncture. Sputum examinations always negative. No autopsy.
769. History: Diagnosis clinical. Fluid from lumbar puncture clear and showed tubercle bacilli on the second examination.
777. History: Breast fed till two months old, then bottle fed. Meningitis confirmed by lumbar puncture.
789. History: Clinical diagnosis only received.
791. History: Clinical diagnosis confirmed by lumbar puncture.
800. History: Clinical diagnosis confirmed by lumbar puncture.
805. History: Clinical diagnosis confirmed by lumbar puncture.
809. History: Clinical diagnosis confirmed by lumbar puncture.
811. History: Clinical diagnosis confirmed by lumbar puncture.
815. History: Clinical diagnosis confirmed by lumbar puncture and presence of tubercle bacilli in sputum.
817. History: Clinical diagnosis confirmed by lumbar puncture.
818. History: Clinical diagnosis confirmed by lumbar puncture.
819. History: Clinical diagnosis confirmed by lumbar puncture.
820. History: Lumbar puncture done post-mortem. Lobar pneumonia and double otitis media also diagnosed on clinical findings.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
525	Wrist Joint	H. Type 1	Non-virulent	R.B.	28 yrs.	F.	6 yrs.	Multiple Tuberculosis of Bones and Joints
526	Hip	"	"	E.M.	8 "	M.	1¼ yrs.	Tuberculosis of Hip
534	"	"	"	J.W.	5 "	M.	3 yrs.	"
558	Pus	H. Type 3	"	A.M.	3 "	F.	3 mos.	Pott's Disease
578	Hip	H. Type 1	"	M.B.	4 "	F.	2 yrs.	Tuberculosis of Hip
586	Knee	"	"	P.F.	8 "	M.	4 mos.	Tuberculosis of Knee
591	Knee	"	"	G.F.	22 mos.	M.	6 "	"
592	Tibia	"	"	H.N.	4 yrs.	M.	6 "	Tuberculosis of Tibia

525. History: Past history negative. Six years ago began to have pain in hip. Physical examination (November 6, 1908): Slight flexion of hip, head of femur absorbed. The sixth to eighth dorsal vertebræ also found involved. Right wrist was operated on January 12, 1909, which healed. On April 6th the left wrist was operated upon. The left knee became involved and was strapped May 8th. Discharged July 6th, improved, wearing plaster corset.

526. History: Paternal grandmother tuberculous. Present illness began October, 1907, after injury to hip; treated by extension and apparently cured. The following June fell again, and since then child has had pain. Operated upon July 18, 1908; in January, 1909, abscess of thigh evacuated. Sinuses still discharging, but child improved.

534. History: Family history negative. At two years was run over. First symptom was pain referred to ankle. On examination left hip found flexed; marked spasm on motion; atrophy of muscles. Extension applied. Several abscesses developed, which were opened. Discharged improved, but sinus still open.

558. History: Father died of tuberculosis. On examination large fluctuating mass over sacro-iliac articulation; resistance on extreme flexion. This was opened. Six months later improved, but sinus still discharging.

578. History: Family history negative. Present illness began one and one-half years ago following a fall which caused limp and pain. Three months later another fall; has limped since. Physical examination (October 19, 1908): Lumps, pain and limited movement in left hip. Operated on November 4th for reduction and extension, on February 16th for abscesses about hip. Discharged April 9th, unimproved.

586. History: Four months ago stated to have had pain in knee. Physical examination shows contractures, ankylosis and induration about left knee. Treated by fixation and traction for few months; then arthrotomy performed and ends of femor and tibia curetted. Discharged three months later, improved.

591. History: Breast fed one month, then cow's milk. About January 1, 1909, had pneumonia and pleurisy; sick about one week. In October, 1908, it was noticed that left knee was swollen. Put up in plaster; no improvement. Later pain and limp developed; swelling increased. Temperature normal till March 1st; then rose to 105.6° and remained high until child died. Operation March 3, 1909; incision, thin fluid and granulation tissue removed from upper part of cavity; no necrotic areas found. Developed pneumonia and died March 7th.

592. History: About six months ago swelling noticed in left leg, which grew gradually worse; ten days ago opened spontaneously. Physical examination (October 18, 1908): At lower end of radius and ulna anteriorly are two depressed scars as if of old sinuses. Tibia found involved near upper epiphysis. Operation October 21, 1908; incision and curetting; on three subsequent occasions granulations and dead bone removed. Wound still unhealed June 11, 1909.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
638	Hip	H. Type 1	Non-virulent	O.S.	8 yrs.	M.	4¼ yrs.	Tuberculosis of Hip and Vertebræ
639	"	"	"	H.R.	7 "	M.	1 yr.	Tuberculosis of Hip
658	"	"	"	C.G.	7 "	F.	4 yrs.	Tuberculosis of Hip (both sides)
662	Knee	"	"	M.L.	8 "	F.	1⅔ "	Tuberculosis of Knee
664	Knee-Hip	"	"	A.B.	4 "	M.	1 yr.	Tuberculosis of Hip and Knee
665	Knee	"	"	M.C.	9 "	M.	2⅙ yrs.	Tuberculosis of Knee
673	Left Metatarsal and Phalanx	"	"	A.M.	20 mos.	M.	2 mos.	Tuberculosis of Metacarpal, Metatarsal Bones and Phalanges
674	Right Metarsal	"	"	A.M.	20 "	M.	2 "	"

638. History: Family history negative. Attack of measles three years ago, when child began to limp and have pain in thigh. Physical examination (January 10, 1908): Unable to walk. Involvement of right hip and dorsal vertebræ, sixth to eleventh. X-ray showed destruction of acetabulum and head of femur. Treated with plaster of paris jacket and spica. On March 15, 1909, abscess found above right groin, which was opened. Discharged May 8, 1909. Sinus still open, improved.

639. History: Family history negative. One year ago pain in left hip and limp began; moderate swelling. Similar symptoms four months ago in left knee. Not able to walk for four months. Physical examination (March 18, 1909): Abscess of left thigh; pain in motion. March 23d, incision of abscess. Discharged May 11th, improved.

658. History: Family history negative. Four years ago began to have pain in right hip, limp and weakness. Abscess developed September, 1908. Symptoms developed in left hip March, 1906. Physical examination (March 30, 1909): Induration and abscess about both hips. Left hip dislocated by extreme adduction. Abscesses opened and curetted April 16, 1909. Present condition improved.

662. History: Family history negative. One year ago knee became swollen and tender. Physical examination (August 11, 1908): Left knee swollen and rotated outward; discharging sinus present. Treated by immobilization April 13, 1909; arthrotomy, patella involved; joint curetted. Discharged June 1, improved.

664. History: Family history negative. One year ago pain developed in left knee, unable to walk for nine months. Two months ago pain began in right hip. Right knee gave some symptoms. Physical examination (January 21, 1909): Abscess of right thigh anteriorly; left knee involved, with abscess on lower outer thigh and sinus posteriorly over lower part of tibia. Incision and drainage of abscess. Other abscesses opened since. Discharged July 6, 1909, improved.

665. History: Family history negative. Delicate child. Two years ago abscesses appeared on back and knee, which have discharged since. Physical examination (March 2, 1909): Sinus of right thigh anteriorly between knee and hip, spinal curvature, tuberculous. Operation, evacuation of abscess on right leg. X-ray shows lesion of right knee. May 11, 1909, patella removed, joint found involved, and pus present. Still in hospital; sinuses open August, 1909.

673. History: Family history negative. About February 10, 1909, swelling noticed in left hand. Child stopped walking and feet began to swell. Dorsal surface of hand and feet became tender. Fever, irritability and restlessness present all the time. Operation April 3d, left hand incised; greenish pus and dead bone found. April 20th, incision and curetting of abscesses of feet. Improved. Developed measles May 2d and was transferred.

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
747	Pus (Hip)	H. Type	Non-virulent	L.O.	10 mos.	F.	3 mos.	Tuberculosis of Hip Pulmonary Tuberculosis

747. History: Fluctuating swelling over hip joint. Aspirated. Thick blood-stained fluid containing many tubercle bacilli. Later examinations showed tubercle bacilli in the sputum.

621. History: Past history negative. About seventeen months ago after birth of child began to have pain in abdomen and hips, nausea, vomiting and diarrhœa. Lost in strength and weight and had occasional sweats. Condition gradually became worse. Physical examination negative except for tender hard mass in posterior fornix. On operation the peritoneum over uterus found congested and studded with tubercles; some clear fluid in peritoneal cavity. Both tubes were removed. Pathological examination showed few fine tubercles in serous coat; lumen full of caseous material.

GENITO-URINARY TUBERCULOSIS

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
621	Fallopian Tube	H. Type 1	Non-virulent	E.L.	24 yrs.	F.	17 mos.	Tuberculous Salpingitis and Peritonitis
649A	Urine (right)	"	"	M.DeD.	Adult	F.	3 "	Tuberculous Kidney
649B	Urine (left)	"	"					
762	Fallopian Tube	"	"	M.S.	35 yrs.	F.	9 wks.	Tuberculous Salpingitis and Peritonitis
763	"	"	Not Tested	P.S.	22 "	F.	4 yrs.?	"
799	"			V.B.	25 "	F.	2 mos.	Tuberculous Salpingitis

649. History: Past history negative. About three months ago started to pass blood in her urine. Has had pain in kidney region for two years; lately has been more intense and accompanied by nausea, loss of appetite, and constipation. Physical examination shows some areas of dullness in right lung. There is a mass on the right side below the liver. Urine was collected by means of segregator, that from left side being amber, cloudy, with marked white muco-purulent deposit; 16 c. c. collected; that from the right side looked like diluted milk; 2 c. c. only collected. A nephrotomy was first performed, later a nephrectomy, of right kidney. Discharged, wound still open.

762. History: Operative case. No other details.

763. History: Operative case. No other details.

799. History: Operative case. No other details.

TUBERCULOSIS OF SKIN

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
746	Skin	H. Type 1	Non-virulent	S.C.	23 yrs.	F.	8 yrs.	Lupus

MISCELLANEOUS

No.	Material	Culture	Rabbit Virulence	Name	Age	Sex	Duration Disease	Diagnosis
661	Pus	H. Type 3	Non-virulent	L.T.	65 yrs.	F.	18 mos.	Tuberculous Abscesses

746. History: Father probably tuberculous. Patient had enlarged cervical nodes at eleven years, which broke down. At fifteen years present trouble began, starting on the nose and face and spreading gradually to the body.

661. Past history negative. Duration of present illness eighteen months; began with pain in right arm. An abscess developed in the right side of chest. This was opened and a resection of rib performed. At present has abscesses on left side of neck over scapula and under right breast and arm. Has had bad cold all winter. Abscesses were incised and drained. There were no abnormal signs in the lungs. Discharged improved.

VIRUSES FROM MILK

No.	Material	Cultures	Rabbit Virulence
561	Milk	B. Type I	Virulent

VIRUSES FROM CATTLE-CONTROLS*

No.	Material	Cultures	Rabbit Virulence
802	Mediastinal Node	B. Type	Not tested
803	"	"	"
804	"	"	"

*Cultural controls for the second series of cases.

For ease of reference to the bovine viruses the following list is added. They are classed as bovine by reason of their cultural characteristics and virulence for rabbits.

Tuberculous adenitis (children)—96, 163, 184, 216, 217, 247, 262, 381, 393, 535, 536, 590, 636, 654, 669, 690-91, 694, 712, 760.

Abdominal tuberculosis (children)—109, 433, 533 and 708-9.

Generalized tuberculosis (children)—62, 63, 69, 66, 122-3, 634, 650, 717-8, and 764.

Tubercular meningitis—678 and 777.

Genito-urinary tuberculosis—199

Elsewhere we have described duplicate strains isolated for different reasons, *viz.*, to control the cultural characteristics of viruses showing variations from different organs of a case for the possibility of double infection, from inoculated calves to see if any change had taken place. As has been noted in a few cases, wide variations in duplicate strains, or even in certain tubes of the same strain, may arise. That this is rare may be seen by comparison of the strains given in the following table with the strains used for all the differential tests. These duplicates were mostly isolated to safeguard against loss, and nothing further than cultural work was done with them, their only value being for comparison.

Diagnosis	No.	Cultures	Notes
Pulmonary Tuberculosis Adults	*11 (a)	H. Type 1	Same as main strain.
	11 (b)	H. Type 1	"
	22 (a)	H. Type 1	"
	22 (b)	H. Type 1	"
	27 (a)	H. Type 1	"
	32 (a)	H. Type 1	"
	38 (a)	H. Type 1	"
	38 (b)	H. Type 1	"
	39 (a)	H. Type 1	"
	40 (a)	H. Type 1	"
	41 (a)	H. Type 1	"
	42 (a)	H. Type 1	"
	44 (a)	H. Type 1	"
	46 (a)	H. Type 1	"
	49 (a)	H. Type 1	"
	53 (a)	H. Type 1	"
	54 (a)	H. Type 1	"
	59 (a)	H. Type 1	"
	60 (a)	H. Type 1	"
	84 (a)	H. Type 1	"
	88 (a)	H. Type 1	"
Tuberculous Adenitis—Adults	31 (a)	H. Type 1	Same as main strain.
Tuberculous Adenitis—Children	7 (a)	H. Type 1	Same as main strain.
	29 (a)	H. Type 1	"
	96 (a)	B. Type 1	"
	116 (a)	H. Type 1	"
	163 (a)	B. Type 1	"
	205 (a)	H. Type 1	"

*The numeral refers to the main strain, a, b, etc., duplicates.

Diagnosis	No.	Cultures	Notes
Tuberculous Adenitis—Children (Cont.)	216 (a)	B. Type 1	Same as main strain.
	536 (a)	B. Type 1	"
	636 (a)	B. Type 1	"
	669 (a)	B. Type 1	"
	694 (a)	B. Type 1	"
Abdominal Tuberculosis—Children	155 (a)	H. Type 1	Same as main strain.
Generalized Tuberculosis—Children	58A(a)	H. Type 1	Same as main strain.
	58B(a)	H. Type 1	"
	62 (a)	B. Type 1	"
	66 (a)	B. Type 1	"
	66 (b)	B. Type 1	"
	69 (a)		
	98 (a)	H. Type 1	"
	99 (a)	H. Type 1	"
	122 (a)	B. Type 1	"
	123 (a)	B. Type 1	"
	123 (b)	B. Type 1	"
	123 (c)	B. Type 1	"
Tubercular Meningitis	4 (a)	H. Type 1	Same as main strain.
	61 (a)	H. Type 1	"
	61 (b)	H. Type 1	"
	72 (a)	H. Type 1	"
Tuberculosis of Bones and Joints	673 (a)	H. Type 1 (?) (potato not planted)	Same as main strain.
Viruses from Milk	8 (a)	B. Type 1	Same as main strain.
	12 (a)	B. Type 1	"
	14 (a)	B. Type 1	"
	89 (a)	B. Type 1	"
Viruses of Bovine Origin	321 (a)	B. Type 1	Same as main strain.

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THE REACTION CURVE IN GLYCERINE BROTH AS AN AID IN DIFFERENTIATING THE BOVINE FROM THE HUMAN TYPE OF TUBERCLE BACILLUS

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From the first the division of tubercle bacilli into a human and a bovine type has been based upon cultural characteristics and virulence for laboratory animals, especially rabbits. While endeavoring to establish further biological differences between the two types Theobald Smith in 1903 called attention to an apparently constant dissimilarity in reaction curves obtained when both types were cultivated on glycerine broth. There was at the start a reduction in the acidity of the medium, but the human type of tubercle bacilli soon produced enough acid to bring the reaction of the medium up to or beyond the original level, while bacilli of the bovine type continued to reduce the acidity until the broth became neutral or even alkaline. Although there was at times a slight acid production following this stage, the final reaction for a bovine virus never approached the initial degree of acidity. Further observations, which Smith published later, tend to illustrate the same difference between the two types.

Relatively few observers have undertaken the study of this reaction curve. Bang obtained results in the main identical with those of Smith. He extended his tests to twelve avian viruses, and observed that they followed the bovine type of curve, but produced more alkali than his bovine controls.

Lewis, at the suggestion of Theobald Smith, studied the reaction curves of six viruses of the human and nine of the bovine type, isolated from fifteen cases of cervical adenitis. For two of these viruses he obtained reversed reaction curves; Vir. 07.8, culturally and in virulence of the bovine type, gave a typical human reaction curve. Vir. 07.54, human in its virulence and cultural characteristics, gave in one test a human curve, in three other tests a curve of the bovine variety. In addition to these, there were four viruses of the bovine type, which in the early generations gave a human reaction curve, but later produced a typical bovine curve.

Duval obtained very atypical results with two of his viruses. These came from cases of a peculiar type of the disease and his results seem to require further proof.

Mohler and Washburn had among their viruses four which gave reversed curves; two of these were of the human and two of the bovine type.

Fibiger and Jensen reported thirty cases, and concluded that, while the majority of human viruses produced one kind of reaction curve and most of the bovine the other, the number of both types of virus giving an intermediate curve was too great to make a division satisfactory. They found that the amount of growth and the acid production were not directly related. This was contrary to results obtained by Beitzke, whose tests, however, are few and rather incomplete.

The Royal Commission found that the final reaction of all the viruses could be so grouped as "to form an unbroken series in which there is nowhere a gap that would justify the conclusion that two essentially different kinds of organisms are dealt with." In the four viruses which produced atypical reaction curves they noted variations in cattle virulence; a bovine curve resulting when the particular strain was highly virulent, a human reaction curve accompanying low virulence. To facilitate observations of the change in reaction, the Royal Commission have used, in addition to glycerine broth, glycerine litmus milk. On this medium all the rapidly growing viruses of the human type produced an initial increase in alkalinity, after which the milk turned acid and coagulated. When growth

was less vigorous the milk became acid, but failed to coagulate. Poorly growing viruses of the bovine type left the milk alkaline, while those which grew well turned it acid, but never coagulated it.

It was thought desirable to test a large number of the viruses which had been isolated at the Research Laboratory from four hundred and seventy-eight unselected cases of tuberculosis in man, and also viruses from cattle, to ascertain how far this variation in acid production could be depended upon to aid in the differentiation of types. The tests were begun after both types had been cultivated on artificial media for some time and had become thoroughly accustomed to them. A few of the viruses, all of the human type, were transferred to glycerine broth after having been cultivated on artificial media for three or four generations only.

Unfermented 1 per cent. peptone beef broth containing 5 per cent. of Merck's glycerine was used as a culture medium; the reaction varied between 1.9 per cent. and 2.4 per cent. acid to phenolphthalein. In the first three sets of tests Lehn and Fink's glycerine has been used; the results seem to be identical.

As soon as a virus had acquired the ability to form a good pellicle on this medium it was planted out in three Erlenmeyer flasks of one hundred cubic centimeters capacity, each containing thirty-five cubic centimeters of the glycerine broth. This amount gives a layer of fluid about 1.5 centimeters deep and offers the largest possible surface for growth. Tin-foil caps proved generally efficient in checking undue evaporation. In looking over the report of other observers one is struck by the fact that no two agree on the length of time during which they continued the tests, nor has any one titrated at regular intervals of time. Thus "end-reactions" are recorded after sixty-five, eighty-nine, or two hundred and twenty-five days. In no instance could a pellicle be induced to grow on either solid or fluid media after ninety days, and it was therefore decided to make this period the end of a test. Titrations of the three flasks were planned so as to use fluid from flask A at the end of fifteen and forty-five days, from flask B at the end of thirty and sixty days, and from A, B, and C at the end of ninety days. The final reaction, therefore, represents the average of the three flasks, except in a few instances where contamination, excessive evaporation, or very poor growth caused a flask to be ruled out. Although two of the flasks for each virus were opened twice during the experiment, the number of contaminated flasks was not large. For about two-thirds of all the viruses tested, the final growth in each flask was collected on dried and weighted filter paper and was weighed, after drying in the incubator for forty-eight hours. As the relative growth of the several viruses was wanted, rather than the absolute weight of any one pellicle, this method was deemed sufficiently accurate. But there was often considerable difference between the actual weight of a pellicle and its estimated growth. The latter is expressed in grades, of which Grades 1-3 indicate poor growth, Grades 4-6 moderate development, Grades 7-9 luxuriant growth.

Of the one hundred and seventy-three viruses which have been cultivated on glycerine broth, fifty-three were tested twice, seven three times and one four times on different lots of broth. Generally speaking, the human viruses have reduced the reaction of the broth to nearly neutral, and later have produced enough acid to bring the reaction back somewhere near the initial. The bovine viruses have rendered the medium alkaline and the final reaction, though often above the lowest reaction recorded, has been considerably lower than the initial acidity of the medium. According to their final reactions both human and bovine viruses may be divided into three groups each, which are not sharply separated, but merge gradually into one another.

Group I of the human type includes by far the largest number of all the human viruses; its reaction curve corresponds with the classical "human curve" of Smith. Judged by the amount of alkali produced during the earlier growth, the viruses of Group I, human type, may again be

divided into three sub-groups, of which HIa is the largest. The viruses included in HIa reduce the acidity of the medium to a point between 0 and 1.0, later raising it above the original. The viruses belonging to HIb produce more alkali, and the reaction of the broth becomes slightly alkaline; later its acidity again increases and finally exceeds the initial per cent. HIc includes those viruses which produce relatively little alkali; the reaction of the broth never falls below 1.0 + and soon rises from this, above the degree of initial acidity.

Group II of the human type is also recorded by Smith. It comprises those viruses which under the same conditions produce twice as much acid as the viruses of Group I. But while Smith found this peculiarity a constant phenomenon of a virus, this has not been our experience throughout, as Table III of duplicate tests shows. According to the greater or less amount of alkali produced in the initial stage, Group II, human type, may be divided into two sub-groups, of which HIIa includes those viruses which do not produce any considerable amount of alkali, while to HIIb belong the viruses which markedly reduce the acidity of the broth, but later manufacture enough acid to double the initial reaction. Two viruses of this sub-group, pulmonary tuberculosis, adults, 485, and general tuberculosis, children, 161, have rendered the broth slightly alkaline.

Group III, human type, is the smallest of the three, but it is of especial interest since its viruses approach most nearly to the bovine type of curve. They first reduce the acidity of the medium, and later produce only enough acid to bring the end reaction back to or slightly below the initial. HIIIa includes those viruses which produce relatively little alkali, while those which during the early stage reduce the reaction of the broth below neutral constitute HIIIb.

The same division into three groups holds for the viruses of the bovine type. Again the largest number belong to Group I, for which the end reaction is somewhat higher than the lowest reaction recorded, but much below the initial acidity; this reaction curve agrees with Smith's classical "bovine curve."

Group II, bovine type, is the next largest, and because of the relatively high end reactions the viruses included in it approach very closely —and in some individual cases even overlap—the reaction curve of the third group of the human type. Group III, bovine type, is very small and comprises those bovine viruses for which the end reaction is the lowest reaction recorded.

In the following table are given the reactions of one virus representative of each group or sub-group:

TABLE I

HIa	Virus 574—Pulmonary Tuberculosis, Adults					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	4	5	6	7
Reaction of broth..	2.2+	1.1+	.2+	1.3+	1.8+	3.1+

HIb	Virus 665—Tuberculosis of Bones and Joints					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	4	6	7	8
Reaction of broth..	2.2+	1.6+	.3+	.7+	.1—	2.8+

TABLE I—Continued

HIc	Virus 692—General Tuberculosis, Children					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	4	6	7	7	8
Reaction of broth..	1.9+	1.2+	2.4+	2.7+	2.9+	3.0+

HIIa	Virus 582—Pulmonary Tuberculosis, Children					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	5	5	5	5
Reaction of broth..	2.1+	1.7+	3.1+	4.2+	3.6+	5.1+

HIIb	Virus 713—General Tuberculosis, Children					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	3	5	6	7
Reaction of broth..	1.9+	1.2+	.2+	2.0+	3.4+	4.0+

HIIIa	Virus 215—Genito-urinary Tuberculosis					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	1	1	2	3	4
Reaction of broth.	1.9+	1.8+	1.8+	1.4+	1.0+	1.8+

HIIIb	Virus 650—General Tuberculosis, Children					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	4	4	5	6	7
Reaction of broth..	2.1+	.6+	0	.5—	.8—	1.6+

TABLE I—Continued

BI	Virus 12—Milk Virus					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	3	4	4	5
Reaction of broth..	1.9+	.3—	.6—	.7—	.5—	.2—

BII	Virus 216—Tuberculous Adenitis, Children					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	3	5	7	7
Reaction of broth..	2.1+	.6+	.3+	.2+	.3+	1.2+

BIII	Virus 491—From Cattle					
Days of growth...	0	15	30	45	60	90
Grade of growth...	0	3	3	5	5	6
Reaction of broth..	2.2+	1.0+	.3+	.4+	.2—	.4—

Table II shows the number of reaction curves included in each group or sub-group, classified according to source of viruses. The viruses numbered 173, the tests 243.

Five human viruses, constituting one set of tests, gave such curious returns that they may well be considered apart. As far as can be ascertained the broth on which they were grown was in all respects like the other lots used throughout these experiments, but on it all the viruses grew poorly and the reaction of the medium at different times was either just above or just below the initial reaction. This is unlike anything noted in our other tests. Smith reports a similar experience and confesses that he is unable to account for it, but warns against using only one lot of broth in determining the type of a virus by the glycerine reaction curve. The fact that in our experience the reaction curve was thus irregular for all the viruses grown on that lot of broth, while duplicate tests of several of the viruses on different lots of broth failed to show anything out of the ordinary might perhaps mean that there had been an error in the preparation of the medium.

Virus 609. Tuberculous adenitis, adults, may be taken to represent these five viruses.

Days of growth...	0	15	30	45	60	90
Grade of growth...	0	2	2	3	3	4
Reaction of broth..	2.4+	2.1+	2.3+	1.8+	2.1+	2.6+

TABLE II

Source	IIIa	HIb	HIc	HIIa	HIIb	HIIIa	HIIIb	Total H	BI	BII	BIII	Total B	Total
Pulmonary tuberculosis, adults..	47	6	29	4	4	3	0	93	2	0	0	2	95
Pulmonary tuberculosis, children.	1	2	3	1	2	0	1	10	0	0	0	0	10
Tuberculous adenitis, adults.....	2	0	2	0	0	0	0	4	0	0	0	0	4
Tuberculous adenitis, children...	10	2	6	1	6	1	0	26	9	5	0	14	40
General tuberculosis, children....	6	1	9	2	5	4	1	28	4	3	1	8	36
Tubercular meningitis, children..	8	2	1	1	0	2	0	14	0	0	0	0	14
Abdominal tuberculosis, children.	0	0	1	0	0	0	0	1	0	0	0	0	1
Genito-urinary tuberculosis.....	0	0	0	0	0	1	0	1	1	0	0	1	2
Tuberculosis of bones and joints.	9	2	1	0	1	0	0	13	3	0	0	3	16
Miscellaneous.....	2	0	0	0	1	0	0	3	0	0	0	0	3
Milk viruses.....	0	0	1*	0	0	0	1*	2*	1	2	0	3	5
Bovine virus from cattle.....	4	0	2	0	0	0	0	6	6	4	1	11	17
	89	15	55	9	9	11	3	201	26	14	2	42	243

*Virus No. 111, human type.

The majority of viruses which were repeatedly tested remained constant to one group of curve; in twenty-two of them, however, nineteen of the human and three of the bovine type, there were variations, the curve falling now into one group, and now into another. In most of these cases the greater acid production went hand in hand with more luxuriant growth, but this was not constant. Thus Virus 583 in one test reached Grade 8 of development, without producing as much acid as in another test, when it failed to develop beyond Grade 4. Virus 536 in one test caused the broth to become .4 acid, while in another test the final reaction reached 1.7+, yet in both tests the growth was Grade 6.

Details of these duplicate tests will be found in Table III, showing reaction curve of nineteen human and three bovine viruses, which on repeated tests gave curves not belonging to the same group.

TABLE III

Virus (Source and Number)	Days of Growth	0	15	30	45	60	90	Group
Pulmonary tuberculosis, adults:								
509 (1)	Grade of growth	0	3	3	5	5	6	
	Reaction of broth	2.1+	1.4+	.4+	1.5+	3.7+	5.7	H2b
509 (2)	Grade of growth	0	1	2	2	3	4	
	Reaction of broth	1.9+	1.7+	.6+	.5+	1.9+	3.1+	HIa
485 (1)	Grade of growth	0	5	7	7	7	7	
	Reaction of broth	2.1+	.3—	4.3+	5.2+	6.2+	7.0+	H2b
485 (2)	Grade of growth	0	2	5	5	6	6	
	Reaction of broth	2.1+	1.5+	3.2+	3.3+	—	3.8+	HIc
437 (1)	Grade of growth	0	3	7	7	7	7	
	Reaction of broth	2.1+	1.2+	1.6+	4.0+	4.5+	5.2+	H2a
437 (2)	Grade of growth	0	1	2	3	5	6	
	Reaction of broth	2.1+	1.5+	1.0+	1.4+	3.0+	2.8+	HIb
545 (1)	Grade of growth	0	2	3	5	6	6	
	Reaction of broth	2.1+	1.8+	1.2+	1.1+	3.1+	5.4+	H2a
545 (2)	Grade of growth	0	1	4	5	6	6	
	Reaction of broth	2.1+	2.0+	2.6+	4.2+	3.8+	4.2+	HIc
595 (1)	Grade of growth	0	2	6	6	7	8	
	Reaction of broth	2.2+	1.5+	.2+	—	3.4+	4.5+	H2b
595 (2)	Grade of growth	0	2	4	5	5	5	
	Reaction of broth	1.9+	1.8+	.7+	1.0+	1.0+	3.2+	HIa
603 (1)	Grade of growth	0	1	1	2	5	8	
	Reaction of broth	2.2+	2.1+	2.1+	.6+	3.0+	3.7+	HIa
603 (2)	Grade of growth	0	0	2	2	2	3	
	Reaction of broth	2.4+	2.2+	.9+	1.5+	1.0+	1.6+	H3a

TABLE III—Continued

Virus (Source and Number)	Days of Growth	0	15	30	45	60	90	Group
Pulmonary tuberculosis, children:								
583 (1)	Grade of growth	0	2	4	5	5	6	
	Reaction of broth	2.1+	1.4+	.5+	1.5+	1.4+	3.5+	H1a
583 (2)	Grade of growth	0	1	2	3	3	4	
	Reaction of broth	1.9+	1.8+	1.5+	.2—	1.2—	3.0+	H2a
583 (3)	Grade of growth	0	3	6	7	7	8	
	Reaction of broth	2.4+	.2+	.6—	.5—	.7—	2.1+	H3b
Tuberculous adenitis, children:								
161 (1)	Grade of growth	0	3	4	6	7	7	
	Reaction of broth	2.2+	1.4+	.3—	.3—	3.8+	4.2+	H1b
161 (2)	Grade of growth	0	4	6	7	7	7	
	Reaction of broth	2.4+	.2—	4.0+	3.4+	4.8+	4.7+	H2b
668 (1)	Grade of growth	0	1	1	2	2	4	
	Reaction of broth	2.2+	2.2+	2.3+	2.2+	2.1+	1.4+	H3a
668 (2)	Grade of growth	0	2	7	7	8	8	
	Reaction of broth	1.9+	.8+	2.3+	2.5+	3.3+	4.1+	H2a
668 (3)	Grade of growth	0	5	5	6	6	7	
	Reaction of broth	2.1+	.3+	2.0+	3.1+	3.5+	4.7+	H2a
682 (1)	Grade of growth	0	4	7	7	8	8	
	Reaction of broth	2.0+	1.3+	2.2+	2.6+	2.5+	4.0	H1c
682 (2)	Grade of growth	0	0	6	6	7	7	
	Reaction of broth	1.9+	1.8+	.3+	1.0+	3.5+	4.4+	H2b
706 (1)	Grade of growth	0	1	2	2	3	4	
	Reaction of broth	2.4+	2.4+	3.0+	1.8+	2.6+	2.3+	H1c
706 (2)	Grade of growth	0	2	4	5	6	6	
	Reaction of broth	2.1+	1.8+	.5+	3.5+	3.9+	1.7+	H2a
536 (1)	Grade of growth	0	1	3	4	5	6	
	Reaction of broth	2.2+	1.9+	.4+	.1—	.5+	.4+	B1
536 (2)	Grade of growth	0	2	6	7	7	8	
	Reaction of broth	1.9+	1.0+	0	0	.4—	1.0+	B2
536 (3)	Grade of growth	0	2	3	4	5	6	
	Reaction of broth	2.1+	1.2+	.4+	.4—	.3—	1.7+	B2
General tuberculosis, children:								
165 (1)	Grade of growth	0	4	4	6	6	7	
	Reaction of broth	2.4+	2.0+	2.5+	3.2+	2.7+	4.0+	H1c
165 (2)	Grade of growth	0	3	6	6	7	7	
	Reaction of broth	2.4+	.6+	3.1+	4.3+	4.1+	4.9+	H2a

TABLE III—CONTINUED

Virus (Source and Number)	Days of Growth	0	15	30	45	60	90	Group
General tuberculosis, children:								
276 (1)	Grade of growth	0	1	2	3	4	5	
	Reaction of broth	1.9+	1.9+	1.4+	0	1.0+	2.7+	H1b.
276 (2)	Grade of growth	0	2	4	5	5	5	
	Reaction of broth	2.1+	1.5+	1.7+	4.4+	5.2+	4.3+	H2a
388 (1)	Grade of growth	0	0	1	2	3	4	
	Reaction of broth	2.4+	2.4+	2.6+	2.1+	1.8+	2.3+	H3a
388 (2)	Grade of growth	0	2	4	5	5	6	
	Reaction of broth	2.4+	1.7+	2.7+	4.4+	3.7+	3.8+	H1c
66 (1)	Grade of growth	0	2	2	3	3	3	
	Reaction of broth	2.1+	1.1+	.3+	.2+	.3—	1.1+	B2
66 (2)	Grade of growth	0	1	2	2	3	4	
	Reaction of broth	1.9+	1.6+	0.2+	1.1+	.4+	.3+	B1
Tubercular meningitis, children:								
61 (1)	Grade of growth	0	3	4	4	6	6	
	Reaction of broth	2.0+	1.2+	2.5	2.8	3.2	4.0	H2a
61 (2)	Grade of growth	0	2	3	4	4	5	
	Reaction of broth	1.9+	.7+	2.2+	2.1+	2.4+	3.2+	H1a
684 (1)	Grade of growth	0	2	2	3	3	4	
	Reaction of broth	1.9+	1.7+	1.0+	.4+	.3+	.9+	H3a
684 (2)	Grade of growth	0	2	3	4	5	5	
	Reaction of broth	2.1+	1.9+	2.1+	.3+	.4+	2.2+	H1a
Tuberculosis of bones and joints:								
664 (1)	Grade of growth	0	5	5	6	6	6	
	Reaction of broth	2.1+	.5+	2.3+	3.8+	4.1+	6.1	H2a
664 (2)	Grade of growth	0	2	4	4	5	6	
	Reaction of broth	1.9+	—	.5+	2.1+	3.5+	3.8+	H1a
Miscellaneous:								
661 (1)	Grade of growth	0	2	3	5	6	7	
	Reaction of broth	2.2+	1.5+	.8+	1.8+	2.8+	3.4	H1a
661 (2)	Grade of growth	0	1	3	5	6	6	
	Reaction of broth	2.4+	.8+	.8+	3.1+	3.7+	4.9	H2a
Milk virus:								
III (1)	Grade of growth	0	2	3	3	4	5	
	Reaction of broth	2.2+	1.7+	1.0+	.1—	0	1.4	H3b
III (2)	Grade of growth	0	1	2	3	4	5	
	Reaction of broth	2.4+	2.7+	1.6+	1.1+	2.4+	3.9+	H1c

TABLE III—Concluded

Virus (Source and Number)	Days of Growth	0	15	30	45	60	90	Group
Bovine virus from cattle:								
491 (1).....	Grade of growth	0	3	3	5	5	6	
	Reaction of broth	2.2+	1.0+	.3+	.4—	.2—	.3—	BIII
491 (2).....	Grade of growth	0	3	4	5	6	6	
	Reaction of broth	2.0+	0	.2—	.3—	0	0	BI

There remain nine viruses, four of the human and five of the bovine type. Of these Virus 122, generalized tuberculosis, children; Virus 247, tuberculous adenitis, children; Virus 323 and Virus 490 from cattle belonged, culturally, to Group B1, that is, they grew poorly on glycerine egg in the first three generations, and growth remained sparse in the first five generations. Growth on glycerine potato in the early generations was also poor. About a year after the viruses had been isolated they grew well, or moderately well, on glycerine broth, reaching Grades 5, 6, and 7. Virus 314, from cattle, belonged culturally to Group B3, that is, in the earlier generations the growth on glycerine egg and potato was slight, but increased rapidly, reaching Grade 7 in the fifth generation, resembling the human viruses in the amount of growth. All five viruses were virulent for cattle, Virus 122 showing a special virulence; but recent experiments with rabbits point to a loss of virulence for Virus 490. Each of these viruses on glycerine broth produced a reaction curve of the human type, as will be seen from the table of reactions following. Virus 122 and Virus 247, however, produced bovine curves in one test each.

Of the four human viruses included in this number, Virus 551, pulmonary tuberculosis, adults, belongs culturally to Group H1; that is, it grew actively in the first three generations on glycerine egg and also on potato, and early showed pigmentation.

Virus 517, pulmonary tuberculosis, adults, culturally belongs to Group H2; its growth on glycerine egg in the first three generations was also abundant, but it lacked the power to grow well on glycerine potato.

Virus 558, tuberculosis of bones and joints, culturally belongs to Group H3. The viruses included in this group grew moderately well on glycerine egg in the first three generations, reaching Grades 4-6, and remained without pigmentation. Growth on glycerine potato = Grades 3-4 in the first four generations.

Virus 377, general tuberculosis, children, belongs culturally to Group H4; on glycerine egg it grew to Grades 4-6 in the early generations, but failed altogether to grow on glycerine potato in the first four generations.

With the exception of Virus 517, which reached Grade 7 on glycerine broth, the viruses grew only fairly well on that medium, reaching Grades 4-6 only. Virus 377 and Virus 517 were tested only once, and gave the bovine type of reaction curve; Virus 551 gave on one test a bovine, on another a human, type of curve, while Virus 558 produced once a human and three times a bovine type of curve.

TABLE IV

Showing Reactions Produced by Viruses which Gave a Reversed Type of Curve

Virus Number and Cultural Type	Days of Growth	0	15	30	45	60	90	Type of Curve
377 H4.....	Grade	0	1	2	2	3	3	
	Reaction	2.1+	1.5+	0	.2—	.6+	.1+	BI
517 H1.....	Grade	0	4	5	6	7	7	
	Reaction	2.1+	0	.1+	.1—	.2—	0	BI
551 H1.....	(1) Grade	0	3	4	4	5	5	
	Reaction	2.2+	.2—	.5—	.4—	.2—	.3—	BI
	(2) Grade	0	1	1	3	6	7	
	Reaction	2.1+	2.0+	1.9+	1.1+	2.8+	3.1+	HIc
588 H3.....	(1) Grade	0	3	3	3	3	4	
	Reaction	2.2+	.3—	.6—	.2—	.6—	.3—	BI
	(2) Grade	0	1	3	3	4	5	
	Reaction	1.9+	1.5+	.3—	.8—	.2—	3.2+	HIb
	(3) Grade	0	0	1	3	4	4	
	Reaction	2.4+	2.4+	.6—	.6—	.3—	.2—	BI
	(4) Grade	0	3	4	5	5	5	
	Reaction	2.1+	.9—	1.0—	.8—	.6—	.3—	BI
122 B1.....	(1) Grade	0	5	5	6	6	7	
	Reaction	2.0+	.6+	.4+	1.0+	.6+	1.8+	HIIIb
	(2) Grade	0	4	6	6	7	7	
	Reaction	1.9+	0	.4—	.1—	0	1.4	BII
323 B1.....	Grade	0	0	1	4	5	6	
	Reaction	2.1+	2.1+	2.6+	.5+	3.5+	3.8+	HIa
490 B1.....	(1) Grade	0	3	4	4	5	5	
	Reaction	2.0+	1.3+	2.8+	.6+	3.0+	2.4+	HIa
	(2) Grade	0	3	4	4	5	5	
	Reaction	1.9+	.6+	2.2+	1.9+	3.0+	3.2+	HIa
	(3) Grade	0	4	5	5	6	6	
	Reaction	2.1+	2.8+	3.2+	4.0+	4.1+	3.9+	HIc
314.....	(1) Grade	0	6	7	7	7	7	
	Reaction	2.4+	.1+	2.3+	1.0+	3.2+	4.2+	HIa
	(2) Grade	0	3	4	4	5	6	
	Reaction	2.4+	2.0+	2.5+	3.4+	3.4+	3.0+	HIc
247.....	(1) Grade	0	0	1	1	2	5	
	Reaction	2.2+	1.9+	1.9+	2.0+	1.3+	.3—	BI
	(2) Grade	0	2	4	5	6	7	
	Reaction	2.1+	1.6+	.5—	.1—	.4—	2.6	HIIIb

An examination of these nine viruses might lead one to conclude that the reaction curve was influenced to a certain extent by the abundance of growth, the poorly growing human viruses tending toward a bovine type of curve and the vigorously growing bovine viruses producing a human type of reaction curve. Such has not been our experience throughout these tests. A number of our bovine viruses have reached Grade 7 of growth, and the weight of the dried pellicle has been equal to and even higher than the average weight of the human viruses, yet their reaction curves have fallen into one of the three bovine groups. On the other hand, some of our human viruses which have produced a high acid end reaction have, when dried, barely exceeded a one hundred milligram weight. In other words, we have not found that the amount of growth and the degree of acid production stand in definite and constant relation to each other. Compare the following table, which shows a combination of high acid production and relatively poor growth in a human virus, and the maximum of growth, without corresponding acid production, in a bovine virus:

TABLE V

Virus	Days	0	15	30	45	60	90
545 (human) Pulmonary Tuberculosis—Adults.....	Grade.	0	2	3	5	6	6
	Reaction.	2.1+	1.8+	1.2+	1.1+	3.1+	5.4+
334 (bovine) from cattle.....	Grade.	0	3	7	7	8	8
	Reaction.	2.1+	.1—	.8—	.4—	.3—	.4—

Why some of these viruses have been constant in producing the opposite type of reaction curve, while others varied in repeated tests, we do not know; every effort was made throughout these tests to keep all conditions absolutely uniform. Most of the other observers have had a similar experience, but few offer any explanation. Smith has thought that it might be due to variations of the glycerine and suggests careful analysis to determine the glycerine consumption and decomposition. Lewis believes that one will always encounter irregular and atypical curves when dealing with recently isolated cultures. This affords an explanation for four of his bovine viruses, which in earlier tests produced human reaction curves and later typical bovine curves. It would not explain the results of some of the other workers who were dealing with older cultures. All nine of our atypical viruses had been cultivated on glycerine media for a year or more. Only one of our bovine viruses, 490, had lost much of its rabbit virulence when it produced a human reaction curve in broth.

The following table shows the viruses reported by different observers, for which the glycerine reaction curve does not correspond with the type established by cultural or virulence characteristics:

TABLE VI

Observer	Virus	Culture	Virulence	Reaction Curve	Remarks
Bang.....	HI	No details given		Bovine	In one of three tests
Mohler and Washburn...	Girl I	Human	Human	Bovine, 3 tests Human, 3 tests	
	Sputum C	Bovine	Bovine	Human	
	Human 33	No details given		Bovine	
	Bovine 3	" "	"	Human, 1 test Bovine, 2 tests	
Fibiger and Jensen.....	5	Human	Bovine	Human	
	12	"	"	Bovine	
	16	"	Human	"	
	17	"	"	"	
	20	Bovine	"	"	
	27	Human	"	"	
Royal Commission*.....	B IX	Bovine	"	Human	
Lewis.....	07.8	"	Bovine	"	
	07.54	Human	Human	Human, 1 test Bovine, 3 tests	
	07.9	Bovine	Bovine	Variable	Human, 2 tests early Bovine, 1 test late
	07.53	"	"	"	Human, 2 tests early Bovine, 1 test late
	07.73	"	"	"	Human, 2 tests early Bovine, 3 tests late
	07.94	"	"	"	Human, 2 tests early Bovine, 3 tests late
Grund.....	377	Human	Human	Bovine	
	517	"	"	"	
	551	"	"	Human, 1 test Bovine, 1 test	
	558	"	"	Human, 1 test Bovine, 3 tests	
	122	Bovine	Bovine	Human, 1 test Bovine, 1 test	
	247	"	"	Human, 1 test Bovine, 1 test	
	314	"	"	Human	
	323	"	"	"	
	490	"	"	"	Lately has lost virulence

*The other three viruses giving irregular curves as noted in their report on the glycerin reaction curve have been excluded because of possible mixture of types. See pages 14 and 15, Final Report, 1911.

Of the thirty-six viruses tested by us which have shown variant characters either culturally or in virulence, only nine were at all irregular in their glycerine reaction curve. Taking away from these eleven the five which have been discussed before, as giving the opposite type of reaction curve, namely, Viruses 377, 517, and 558 of the human and Viruses 122 and 314 of the bovine type, there remain four, all of the human type, Viruses 437, 485, and 545, pulmonary tuberculosis, adults; Viruses 434 and 689, general tuberculosis, children, and Virus 661, miscellaneous lesions.

Four of these, Viruses 434, 545, 434, 661, grew rather poorly in early generations, while Viruses 485 and 689 showed a very slightly increased tendency to produce more generalized tuberculosis in rabbits. These peculiarities indicate, if anything, a leaning toward the bovine type, yet all four of these viruses belong, as far as their reaction curve in glycerine broth is concerned, to Group HI, which is the typical human curve, or to Group II, human type, the viruses of which are distinguished by their high acid end reactions, their only irregularity being the fact that in repeated tests they give reaction curves belonging now to one and now to another group. None of the thirteen viruses included in Group HIII, with very low end reactions approaching the bovine type of curve, showed irregularities either culturally or in regard to virulence.

TABLE VII

Showing Glycerin Reaction Curves of the Four Viruses Which Showed Variations in Cultural or Virulence Characteristics

Virus	Days	0	15	30	45	60	90	Culture	Virulence
437....	(1) Grade	0	3	7	7	7	7	Growth slow on potato. Otherwise typical	Typical
	Reaction	2.1+	1.2+	1.6+	4.0+	4.5+	5.2+		
437....	(2) Grade	0	1	2	3	5	6		
	Reaction	2.1+	1.5+	1.0+	1.4+	3.0+	2.8+		
485....	(1) Grade	0	5	7	7	7	7	Typical	Slightly increased for rabbits
	Reaction	2.1+	.3—	4.3+	5.2+	6.2+	7.0		
485....	(2) Grade	0	2	5	5	6	6		
	Reaction	2.1+	1.5+	3.2+	3.3+	—	3.8+		
545....	(1) Grade	0	2	3	5	6	6	Poor on potato	Typical
	Reaction	2.1+	1.8+	1.2+	1.1+	3.1+	5.1+		
545....	(2) Grade	0	1	4	5	6	6		
	Reaction	2.1+	2.0+	2.6+	4.2+	3.8+	4.2+		
661....	(1) Grade	0	2	3	5	6	7	Moderate growth on all media	Typical
	Reaction	2.2+	1.5+	.8+	1.8+	2.8+	3.4+		
661....	(2) Grade	0	1	3	5	6	6		
	Reaction	2.4+	.8+	.8+	3.1+	3.7+	4.9+		

Concerning our one bacillus of the avian type, it was with difficulty induced to grow on glycerine broth and has therefore been tested once only. Its curve shows the same general direction as that of the bovine viruses, but it does not produce as much alkali. Bang's twelve avian viruses produced more alkali than his bovine controls, but that is apparently due to the fact that his observations extend over a longer period than ours. At the end of ninety days his avian cultures are still more acid than the bovine controls.

A few other acid-fast organisms were included in our tests, and so was a subculture of the original T. B. Koch. Both the timothy and the smegma bacillus reacted like the bovine type of virus, the latter producing more alkali than any other bacillus tested. The curve of T. B. Koch was rather irregular, following at first the human type, but later it again pro-

duced some alkali and left the medium nearly neutral at the end of ninety days.

On glycerinated litmus milk ninety tests have been carried out, with seventy-eight viruses of the human and twelve of the bovine type. The results have corresponded fairly well with those obtained in broth. But pellicle formation on the milk was in many cases delayed, and not once as vigorous as on broth. Thirteen of our human viruses, which grew poorly on the milk, did not produce acid; this includes the atypical human Viruses 551 and 558; Viruses 377 and 517 have not been grown on milk. The two atypical bovine Viruses 314 and 490 turned the milk acid, but did not coagulate it.

CONCLUSIONS

Broadly speaking, the reaction curve in glycerine broth divides tubercle bacilli into two types. The bacilli which possess a low degree of virulence for rabbits and the power to grow well on glycerine media in the early generations produce one type of reaction curve, while those which are virulent for rabbits and which in the early generations grow slowly and with difficulty on glycerine media form the other type of curve in glycerine broth. These two types of glycerine reaction curve are again divisible into groups according to their final reactions. The curves of adjacent groups show much the same general direction and there is a gradation from one group to the next; but the reaction curves of the groups at both extremes are widely divergent. When any large number of viruses is examined there will be found a small percentage of cases which, by cultural characteristics and virulence, belong to one type of tubercle bacilli, while they would be classed with the opposite type of bacilli if judged by their glycerine reaction curve alone. On repeated tests this reversed glycerine reaction curve may, or may not, be a constant feature of these particular viruses, although the conditions under which they have been cultivated are apparently the same in the several tests. Undetected variations of the culture medium must be taken into consideration; it is not advisable to depend on the reaction curve obtained from one lot of broth only, but several examinations of a virus are desirable. In from thirty to forty per cent. of the viruses retested the reaction curves belong to different groups, that is, the end reaction may be high in one test and low or medium in the next. In only three instances was the variation so great as to justify the classification of the reaction curves into different types. In about half the cases the degree of acidity produced has been in direct ratio to the amount of growth.

There is also no constant relation between irregularities of culture and virulence on the one hand and irregularity of the glycerine reaction curve on the other. Some viruses which culturally and in virulence showed nothing unusual have given very atypical curves, while perfectly normal reaction curves were produced by viruses which from cultural and virulence tests could not be called quite typical.

The glycerine reaction curve is undoubtedly a valuable corroborative evidence of a division of tubercle bacilli into two types. Its value is lessened, however, by the number of irregular and atypical reactions encountered, while as a practical aid in determining the type of an individual virus it is also much handicapped by the length of time required to carry it out.

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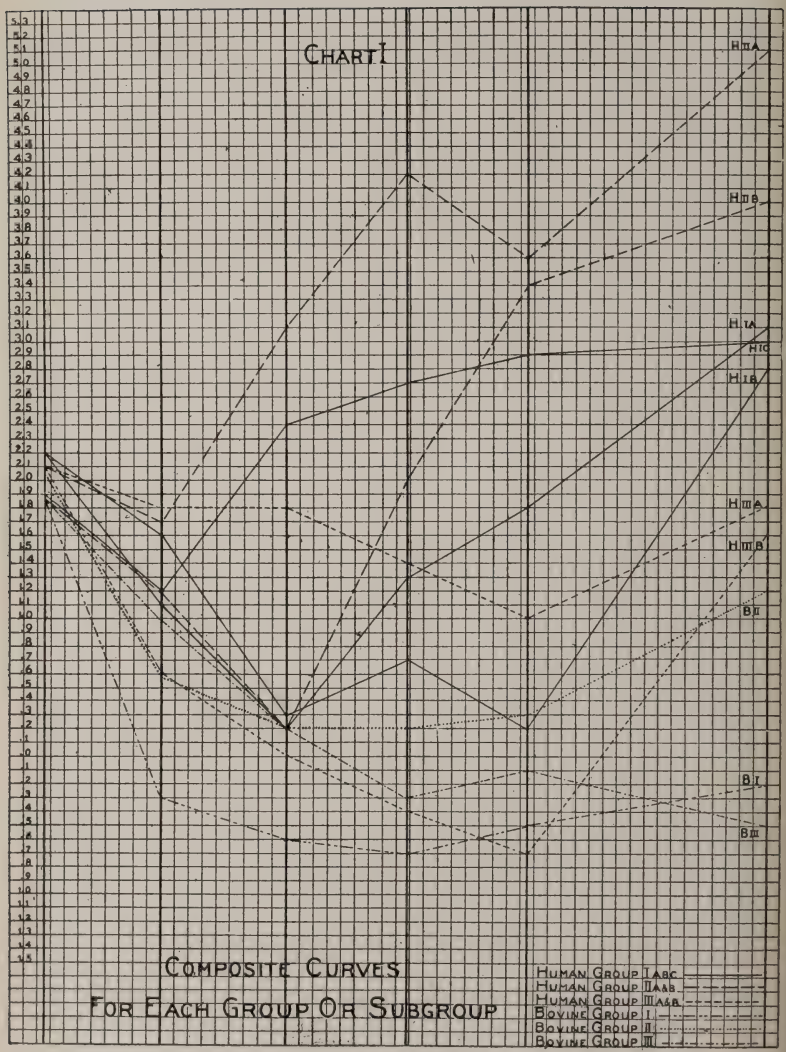
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CHART I
H III A
H II B
H I A
H I C
H I B
H III A'
H III B'
B II
B I
B III
COMPOSITE CURVES
FOR EACH GROUP OR SUBGROUP
HUMAN GROUP I ABC
HUMAN GROUP II A&B
HUMAN GROUP III A&B
BOVINE GROUP I
BOVINE GROUP II
BOVINE GROUP III

THE RESISTANCE OF TUBERCLE BACILLI TO DRY HEAT

CHARLES KRUMWIEDE, JR., M. D.

Although as a general rule all bacteria are more resistant to heat when dry, the degree of resistance of the tubercle bacillus to dry heat as given seems very great. Thus, Schill & Fischer (Mittheilungen. a. d. kaiserlichen Gesundheitsamte, 1884, Bd. II, 131) used dried sputum. Sputum dried 98 or 142 days failed to infect pigs after one hour's heating. A sputum dried only five days infected all the pigs after one-half hour's heating. After one hour's heating only one of three pigs became tuberculous.

Grancher and Ledoux-Lebard (Archives de Médecine Experimentale, 1892, IV, I) conclude from their experiments that heating to 100° C. for two or three hours reduces the virulence of cultures used, but does not destroy it. A comparison of these two reports shows little agreement. A repetition of the work was therefore advisable.

The material for the test was from different recently isolated human cultures (8) on glycerine egg. The cultures were about three weeks old. The bacilli were removed and mixed and ground up in an agate mortar. They were then dried in a thin layer in the incubator * for twenty-four hours and again ground up in the mortar to a fine dust-like condition. This grinding was sufficient to break up clumps of any size, but not sufficient to break up the bacilli. The material was placed in narrow test tubes, which were then drawn out to capillary size and bent at the upper end in an acute angle. Approximately 0.025 gm. of culture was added to each tube. The culture was, therefore, in the bulb-like extremity of the tube, the capillary part giving free exit of air and the upper end, the diameter of the original test tube, but bent down, was plugged with absorbent cotton. A large pail of water was raised to the boiling point and the bulbs suspended in the water by hooking the bent tube over the side of the pail. The bulbs were protected from the side of the pail by a thick layer of cotton. The openings of the tubes being bent downward were protected from the steam which rose upward due to the draft in the hood. A thermometer suspended in the water registered 100° C. The same thermometer, when put into a tube of the same size as used for the tests, registered about 0.20 less. As this tube was open, one is safe in assuming that in the tubes with a capillary opening only the temperature was practically 100° C.

After heating the bacilli were suspended in saline and injected intramuscularly in guinea pigs. The results are given in the following table:

Time	Pig. A.	Pig. B.	Notes
0 control.....	++++	++++	Marked generalized tuberculosis
5 minutes....	++++	+++	Approximately same as control
10 minutes...	++++	++++	Approximately same as control
20 minutes...	++	++	Lesion decidedly less than in control
45 minutes...	——	died acutely	No local caseation at site of injection
1 hr., 15 minutes.....	——	——	No local caseation at site of injection

* The object was to approximate practical dry-air sterilization with air-dried contaminated material.

Evidently there was already some diminution in the number of bacilli at twenty minutes. Complete destruction, however, took place between twenty and forty-five minutes.

Air-dried tubercle bacilli are, therefore, many times more resistant to dry heat than are bacilli heated in fluids or steam.

OBSERVATIONS ON THE ANTIFORMIN METHOD FOR THE DETECTION OF TUBERCLE BACILLI AND THE VALUE OF THE HERMAN STAIN

CHARLES KRUMWIEDE, JR., M. D., AND LOUISA P. BLACKBURN

Numerous efforts have been made to perfect a method by which sputum, negative for tubercle bacilli by direct smear, could be fluidified so that subsequent sedimentation, by gravity or centrifuge, would concentrate the bacilli if present and render detection easier. No satisfactory method had been evolved till Uhlenhuth[1] in 1908 advised the use of antiformin, a hypochloric solution with sodium hydrate added, as the solvent agent. While testing its disinfectant and solvent powers he found that not only were nearly all bacteria quickly killed and dissolved by it, but numerous organic substances were attacked. On trying its effect on tuberculous sputum this was found to be quickly fluidified, but within limits neither the staining properties nor the viability of the tubercle bacilli were affected. In general the following method has been used, the variations being in time of action and strength of antiformin used:

The sputum is mixed with antiformin so that the total strength of the latter is 15 per cent. After vigorous shaking the mixture is allowed to stand until completely fluidified, which will depend on the character of the sputum. The specific gravity of the mixture is then reduced, if necessary, by adding water or alcohol to facilitate sedimentation and centrifuged for one-half hour. The supernatant fluid is then poured off and the sediment shaken up in water and recentrifuged. This washing is necessary, as otherwise the material does not spread well on the slide and staining is interfered with. After pouring off the water smears are made from the sediment and stained. The results as reported by Uhlenhuth, Hune,[2] Thilenius,[3] Seeman,[4] and Meyer[5] have been very good. The last reported 14 per cent. positive results with negative sputa, in some instances the sputum for twenty hours being used and only three to four bacilli found.

Previous to the introduction of antiformin Lange and Nitsche[6] had found that if a hydrocarbon be added to fluidified sputum and thoroughly shaken, as the hydrocarbon rises again it carries up the tubercle bacilli which will be found at the junction of the two fluids when the emulsion clears. They suggested the use of ligroin and advised this method as a substitute for centrifuging. The clearing of the emulsion and rise of the ligroin are aided by standing the mixture in the incubator or in a water bath at 60° C. Smears are made by taking repeated loopsful of material at the junction of the two fluids and placing them on the same spot on a warmed slide. Practically nothing stains except tubercle bacilli.

Haserodt[7] and later Bernhard[8] and Jacobson[9] applied the ligroin procedure to the antiformin-treated sputum with good results. Haserodt used both methods on 300 negative sputa with 7 per cent. positive results. On the ground of comparative results with 20 sputa prepared by mixing different samples, he comes to the conclusion that the ligroin method is more favorable than centrifuging.

We have examined the following specimens of sputa to judge of the practicability of the antiformin method as a routine method for diagnosis and incidentally to compare the crystal violet (Herman[10]) and carbol-fuchsin stains.

Twenty-eight sputa considered negative by the diagnostic laboratory and five sputa containing varying numbers of tubercle bacilli were examined. The method consisted in diluting antiformin with two parts of water, adding an equal volume to the sputum, shaking a few times and allowing the sputum antiformin mixture to stand overnight. The resulting homogeneous mixture was then treated as already described, using the centrifuge method.

The carbolfuchsin stained smears were decolorized and counterstained in Pappenheim's solution; the crystal violet stained smears were decolorized in 10 per cent. nitric acid, rinsed in alcohol and counterstained in Bismarck brown. Where smears are said to have been restained with carbolfuchsin the heating was prolonged to about three minutes, the slide decolorized as in the crystal violet method, and simply dipped in methylene blue (1 per cent.) to render the counterstain very light.

In all cases the duplicate slides were made from the same material, carrying it from slide to slide, with careful mixing rendering them as uniform as possible. We have considered these slides as identical, which naturally cannot be the case, but the final average would rule out accidental variations in the individual slides.

Of the twenty-eight negative sputa examined two were positive on less than five minutes' search in slides made directly from the sputum. Of the remaining twenty-six four were positive in the sediment alone, that is, 15 per cent. positive results with the antiformin.

The first two sputa were also negative with us, after painstaking search of carbolfuchsin preparations. In one case the sediment was also negative with the carbolfuchsin stain, but very numerous with the crystal violet stain. All the carbolfuchsin slides were then restained and the bacilli easily found, though even then the preparations were not quite as good as those stained with crystal violet. In other words, if these examinations had been controlled with crystal violet stain, a few minutes' search would have shown them positive.

Of the four specimens which were positive in the sediment alone, the carbolfuchsin stain failed, though the crystal violet stain revealed bacilli. On restaining the carbolfuchsin slides, however, bacilli were found in three of the specimens, though decidedly fewer in number. The results in the fourth specimen were probably accidental, due to difference in number of bacilli on the individual slides. This leaves no doubt as to which is the better stain.

Five control specimens were examined to confirm the above findings and to see the relative merits of slight modifications of technic.

In three specimens the concentration was over one hundredfold in the sediment. Comparative counts showed more bacilli in the sputum stained with crystal violet, and in the sediment the carbolfuchsin slides were very poor and required restaining. The use of alcohol to reduce the specific gravity of the antiformin sputum mixture was tried and gave better sedimentation and clearer and better stained preparations.

The ligroin method was tried both on positive and negative sputa. The results in the few instances we have tried it have not been as good as the centrifuge method.

The amount of sputum used in the centrifuge method has been from 3 to 7 c. c.

From the comparative work done we conclude that every sputum should be examined with the crystal violet stain, if negative with carbolfuchsin. Further, that as a routine procedure one volume of the antiformin sputum mixture to one or two volumes of alcohol, depending on the character of the mixture, will give the best results, and in the ordinary centrifuge tube will give sufficient sediment for two slides, which, if stained with crystal violet and found negative, can be considered negative. Larger volumes of sputum would give better results probably, but more smears would be required and the time consumed would be prohibitive except in special instances. The guinea pig would be less trouble if slower.

As to the difficulty with the carbolfuchsin stain, possibly two factors are at work, the decolorizing action of methylene blue, and overstaining and blotting out of fuchsin stained bacilli. Very light staining with methylene blue, just sufficient to clear the field and give a contrast, should be done. If other organisms are to be observed, a second preparation had better be made.

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ADDENDA.—The ease with which bacilli were found in two of the sputa using the crystal violet stain, after negative results with carbolfuchsin; and the same result noticed in the examination of smears from the tubercles in the lungs of a case of general miliary tuberculosis raised the following question, Does this occur as frequently as the above small series would lead one to believe? We decided that if they do occur frequently a five minute search of duplicate slides would surely reveal any such marked differences as have been noticed. For this reason the following scheme was followed:

From the specimens of sputa received at the Diagnostic Laboratory Dr. Taylor made two smears. One he stained with carbolfuchsin and sent us the duplicate slide of those which he found negative after five minutes' search. These we stained with crystal violet and likewise searched for five minutes. The results were identical, that is, all were negative with both stains. In this way 135 specimens of sputa were examined.

This does not answer the question, but it at least shows that marked differences are decidedly less frequent than would appear from the smaller series examined. It does not, however, alter our preference for the crystal violet stain. The light counterstaining after carbolfuchsin undoubtedly increases the efficiency of this stain. In spite of this the crystal violet stain gives the better contrast, the field is clearer, the organisms more brilliantly revealed and on comparative counts greater in number. The one possible objection to the stain, *viz.*, the tendency to decolorize at times with vigorous treatment with alcohol, is partly removed by the observation of Van Giesen. He found that if the slide is placed in Gram's solution after decolorization with the acid the dye is fixed and protected from subsequent action of the alcohol. This is more marked in the granules, and especially so if the alcohol treatment be prolonged. The body then becomes faint, but the granules are brilliant violet.

Dr. Grund examined smears from 24 sputa and 24 cultures to compare the decolorizing action of alcohol, exposing one-half the smear to the action of Gram's solution. In three specimens of sputa exposure to alcohol for 16 to 19 hours decolorized both halves of the slide. The bacilli were present in the sputa when only exposed for a short period, though in moderate numbers. In seven sputa the non-gram-treated half was completely decolorized. Of the remaining sputa, with one or two exceptions, the gram-treated half was on the average more deeply stained and this was mainly evident in the granules. On restaining the half not treated by Gram comparison showed that the Gram solution had not completely protected in each instance; the organisms were usually more numerous in the restained half. Careful examination of the half treated with Gram showed very faint bacilli, many taking the counterstain.

The conclusion one may draw from this is that, using the Herman stain, the granules of the tubercle bacilli are the more alcohol-fast portion. The alcohol-fast character is relatively, but not absolutely, increased by the use of Gram's solution.

Practically the results are not as striking as one would have thought from the first series. There is, however, a distinct advantage in the use of the crystal violet stain either as a substitute or control for the carbol-fuchsin method. The use of the Gram's solution should be adopted in using the former stain.

ACID-FAST PARTICLES ASSOCIATED WITH THE TUBERCLE BACILLUS

Ira Van Giesen, M. D.

This is a brief note giving the results and conclusions of a minor study of acid-fast particles associated with and subject to confusion with the tubercle bacillus, both in sputum and in tissues. These particles simulating fragments of the tubercle bacillus are of rather rare occurrence in casual examination of sputum with Ziehl's method, but in systematic examinations of large numbers of sputa in sanatoria the acid-fast particles appear quite frequently, say in 5 per cent. of the daily examinations. The particles give precisely the same reaction in Ziehl's method of staining as the tubercle bacillus itself and apparently have the same degree of acid-fastness in retaining the dye. The particles, as a rule, take the form of minute spherical or slightly elongated points like tubercle bacilli in miniature, or show in various intermediate stages up to larger and coarser and sometimes budding tiny droplets of the same brilliant red hue as the tubercle bacilli. These particles occur quite uniformly or in more or less densely packed spherical aggregations inclosed often within a rim or halo made by their delimitation from the surrounding material of the sputum. Struck by the peculiarity of the inclosure of the particles as if in a hollow spherical envelope, and by their exceedingly capricious appearance in sputum, sometimes suddenly appearing in and vanishing equally abruptly from persistently negative sputum, and noting, also, the fact that these acid-fast particles failed to infect guinea pigs or to develop in appropriate culture media, the idea was entertained that such particles might be fatty food globules making their way into the sputum when coughed up immediately after a meal. This probatory conjecture for the explanation of the particles turned out to be correct, for in examining various fatty elements of food, such as milk, fatty soups, and raw oily foods, precisely similar structures were seen under the microscope when stained with Ziehl's method and decolorized with acid alcohol or Pappenheim's rosolic acid solution. These fatty foods then, especially milk, are liable to contaminate sputum in a highly irregular fashion and to appear as if they were fragments of or aberrant developmental phases of real tubercle bacilli.

In old cheesy material, notoriously difficult in a demonstration of tubercle bacilli, the same mistake is liable to be made, for this material, as might easily be demonstrated by osmic acid and other staining methods, contains numerous fat droplets and probably a variety of the fatty acids and derivates. Among these are certain members of the fatty series the particles of which are acid-fast and may appear under the guise of derivates of the tubercle bacillus, as exhibited often in bovine lymph node cheesy material.

Fæces, containing as they do palmitic, stearic, and some eight other fatty acids, show these acid-fast fatty particles quite frequently.

The importance of recognizing the fatty character of these confusing acid-fast particles associated with the tubercle bacillus is obvious in practical laboratory diagnosis in the examination of sputum, fæces, abscesses, and the like. Besides the possibility of diagnostic errors in cheesy material the proper appreciation of these particles is additionally important because of the mistakes in the literature of the degenerative and reproductive cycles of the tubercle bacillus, which has recently received a fresh and voluminous accession under the stimulus of Much's dubious work on the subject. Schrön,[1] D'Arrigo,[2] Spengler,[3] Walsham,[4] Muir and Ritchie,[5] and others seem wholly averse to the consideration of the real nature of these particles which so readily masquerade in the false rôle of conjectural "capsules," "granules," and "splitter," supposed to represent degenerative and reproductive phases of the tubercle bacillus.

It is not within the plan of this brief initial communication to give a critical analysis of the literature in which these confusing acid-fast particles have been mistaken for phases of the tubercle bacillus. I wish merely at present to express the conviction from certain experiments and studies, which may be detailed later, that these particles may be the subjects of mistakes in diagnosis practically, and their misapprehension a source of confusion in the life history of the bacillus theoretically.

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THE SUBSEQUENT HEALTH OF CHILDREN WHO DRANK MILK CONTAINING TUBERCLE BACILLI

Alfred F. Hess, M. D.

Three years have passed since I undertook an investigation of the milk sold in New York City to determine primarily to what extent the ordinary milk sold in cans, which forms the major part of the milk supply of the city, was contaminated by tubercle bacilli. This study included also an inquiry into the types of these bacilli, whether bovine or human, and a preliminary investigation into the welfare of the children who were so unfortunate as to drink this milk in its raw state. This report was read [1] in 1908, and was afterward published in The Journal.[2] It showed that of the 107 specimens of milk tested on guinea pigs, 16 per cent. contained virulent tubercle bacilli. Details of the report will be found in the original paper. In order to elucidate what follows, however, it seems necessary to review briefly some of the facts as to the method pursued in the investigation. To obtain the specimens I accompanied the municipal milk inspector on his regular tour, and asked the dealer in each store (they were situated mainly in the tenement house district) whether he had small children, and whether the children drank the milk in the raw state. Unless he told me that this was the case I did not take a sample for the test. Of the samples which were found to contain tubercle bacilli, ten were from dealers whose children had been exposed in this way to infection. There were eighteen children in all; nine of these were two years of age or under, and one five years of age. The preliminary study of the health of these children was not conclusive, and the data are summarized in Table 1. This table shows that the health of the children did not seem to have suffered from their exposure, except possibly in one instance, in which a cervical adenitis had developed. In this case, however, no search for tubercle bacilli was made when the gland was incised, and accordingly the nature of the infection was doubtful. The child was poorly nourished and gave a positive tuberculin reaction, as did several of the other children.

Since 1907-1908, a period of over three years, I have continued to supervise the health of these eighteen children, visiting them in their homes about every three months and watching carefully for the development of any sign of tuberculosis. This has been attended with some difficulty, as the people of the tenements in New York move frequently, and seem by habit to leave no address. Sixteen children, however, have been closely followed, one for two years, and only one was completely lost sight of. I realize that the number of children on whose condition I can report is not large, and that a period of more than three years might be of advantage in judging of the development of tuberculous infection; but I make this report now, as I may be unable to follow these cases any longer, and as I believe that the results are of interest from several points of view.

I may add that the only similar report has been a recent one from the Imperial Health Commission of Germany,[3] which investigated the health of 151 children who drank raw milk coming from cows with tuberculosis of the udder. Two of these children between the ages of one and two years developed cervical gland tuberculosis, from which a bovine type of bacillus was cultivated. Some other children showed signs suspicious of tuberculosis. The two tuberculous children drank the infected milk for long periods, but, except for the glandular infection, were in good health one and one-half and two years later, respectively.

This report is open to one criticism, which cannot be made in regard to the investigation which I report. Tubercle bacilli were not demonstrated in the milk. A clinical diagnosis of tuberculosis of the udder of the cow was considered the equivalent of finding tubercle bacilli in the

1. Tr. Sixth Internat. Cong. Tuberc., iv, Part 2.
2. The Incidence of Tubercle Bacilli in New York City Milk, with a Study of Its Effects on a Series of Children, *The Journal A. M. A.*, March 27, 1909, p. 1011.
3. Kossel, H.: Deutsch. med. Wchnschr. 1910, No. 8, p. 349.

milk. Now, although this is generally the case, nevertheless it has been found that the milk from cows with tuberculosis of the udder does not at all times contain tubercle bacilli; and, in the second place, udder tuberculosis is frequently confounded with non-specific infections. It is therefore quite possible that some of these children did not drink milk containing tubercle bacilli.

Of the seventeen cases which I have followed in only one has tuberculosis developed. The other children present the picture of the average tenement house child, most of them poorly nourished, undeveloped, but free from any active tuberculosis. Four have passed through an attack of measles, one has had scarlet fever, two have suffered from pneumonia without inducing tuberculosis. The child who did develop tuberculosis was two years of age when she was given the milk containing tubercle bacilli. This milk was of very poor quality, for milk from the same source was repeatedly found by me to contain virulent tubercle bacilli. In Table 1 this child is described as being poorly nourished, having had cervical adenitis four months previously, showing a positive Von Pirquet reaction, but with a family history negative as regards tuberculosis. I was out of the city when the cervical adenitis developed and the gland was incised, and accordingly missed the opportunity of obtaining some of the pus or tissue for bacteriologic study. Last winter, however, a cervical gland enlarged near the site of incision of the former gland, and in due course showed

TABLE I

DATA CONCERNING CHILDREN WHO DRANK MILK CONTAINING TUBERCLE BACILLI

Milk Dealer	No. of Children	Age Years	Conjunctival Test	Condition One Year Later
A	2	1	—	Healthy
		3	—	Healthy
B	1	1½	—	Healthy
C	2	2	—	Poorly nourished
		4	—	Healthy
W	3	$\frac{5}{12}$	0	Healthy
		2½	—	
		5	+	Poorly nourished. Physical exam. and family history doubtful
E	1	½	—	
B		2	+	Poorly nourished. Cervical adenitis incised four months ago. Von Pirquet positive. Family history negative.
	3	3	—	Healthy
		5	—	Healthy
		9	+	Healthy. Family history negative.
B	1	$\frac{5}{6}$	0	Healthy
B	2	1½	—	Poorly nourished
		3½	+	Healthy. Physical exam. and family history negative.
O	2	2	—	Healthy
		4	—	Healthy
	18		4	

+ = positive reaction.
— = no reaction.
0 = no test.

fluctuation and was incised. This time I obtained some of the pus, which showed no tubercle bacilli in smear preparations, even with the aid of the alkaline sodium hypochlorite ("antiformin") method, but produced tuberculosis in both guinea pigs into which it was inoculated. Without going into detail as to the method of isolating and differentiating tubercle bacilli, for such description is no longer necessary, I may state that the strain proved to be typically bovine, as judged by its cultural characteristics and virulence for rabbits.

There is no means of knowing whether this infection was due solely to drinking in tubercle bacilli on this particular day, or whether this child had been exposed similarly on previous days. We may be certain, however, that infection did not result from a subsequent milk exposure, as the parents were warned of the danger, and henceforth boiled the milk for the children. In this connection it should be noted that it is by no means inconsequential whether a definite amount of tubercle bacilli is ingested in one dose or in repeated doses. The chance of infection is less when the bacilli are taken in at one occasion than when approximately the same number is ingested in divided doses. I was able to demonstrate this fact conclusively by a simple experiment on guinea pigs. It had been

TABLE II

Table of Guinea-Pigs Fed with Tubercle Bacilli in Divided Doses

Experiment	No. of Feedings	Amount of Culture, mg.*		Total Dose, mg.	Result
1	10	0.1	=	1.0	Negative
2	10	0.1	=	1.0	Negative
3	25	0.05	=	1.25	Negative
4	25	0.05	=	1.25	Tb.
5	30	0.05	=	1.50	?
6	30	0.05	=	1.50	Tb.
7	30	0.05	=	1.50	Tb.
8	35	0.05	=	1.75	Tb.

*Ten guinea-pigs, used as a control experiment, received 2 mg. of this culture in one dose, and failed to develop tuberculosis.

ascertained that 2 mg. of a certain bovine culture of tubercle bacilli did not produce tuberculosis in guinea pigs when given to them suspended in milk. Of ten pigs which had received this amount none developed tuberculosis. The animals were fed by means of a medicine dropper, and the suspension was so calculated that each pig received about ten drops of milk. In a second series ten pigs, weighing between 250 and 350 gm., were now given less than 2 mg. (which may be called the subinfectious dose of this culture), but instead of being fed to them at one time, this amount was divided into ten, twenty, thirty and thirty-five doses, given on as many days. Two of the pigs died prematurely; the other eight were killed after three months and were examined carefully for tuberculosis (Table 2). From the table it will be seen that several of the animals developed tuberculosis. In other words, a quantity of tubercle bacilli which may be insufficient to cause infection when given at one time may have this power when given frequently in small amounts. Repeated exposure to infection at short intervals tends to lower an animal's resistance, a law which probably applies to other bacteria besides tubercle bacilli.

This same phenomenon may well have played a part in the infection of the child who developed tuberculous adenitis. The milk which she drank was found to contain tubercle bacilli oftener than any other which I examined, and it is probable that she consumed tubercle bacilli on many occasions, and that the summation of exposures resulted in infection.

From a clinical point of view there are some points in this case worthy of passing mention: The bovine bacilli remained latent in the cervical glands for two years and caused merely a slight enlargement; the tonsils were not markedly hypertrophied, nor were adenoids present; and, finally, the general condition of the child decidedly improved during the course of the past year and the cutaneous tuberculin reaction became much more pronounced.

Without exaggerating the importance of the bovine bacillus in the etiology of tuberculous lesions, I feel that we must acknowledge that its rôle is not insignificant in childhood. The case of milk infection which I report is not of great interest as an isolated instance of infection by this type of bacillus. Since the report of the British Tuberculosis Commission and the more recent report of Park and Krumwiede[4] showing the frequent occurrence of bovine infection among children, it is unnecessary to adduce further evidence as to the incidence of this type of tuberculous infection. It was rather my purpose to show by means of a definite case that, although a group of children can frequently, without apparent harm, drink milk containing tubercle bacilli, nevertheless such milk can be shown at times to incite infection.

I cannot close without some words as to the means of safeguarding the children against infection by the bovine tubercle bacillus. I feel that I can repeat what I wrote in my previous paper: "Milk not coming from tuberculin-tested cows should be pasteurized or brought to a boil." In substance this is the position recently taken by the New York City Board of Health as regards milk for infants. I should like to add to what I said previously that I believe some care should be taken also in regard to butter, which, it is needless to say, is frequently permeated with virulent tubercle bacilli. Butter is fed without concern to almost all children over one year of age. It is a strange anomaly that we should fear the tubercle bacillus in milk, but urge our children to take butter, which has been found so often to harbor the same bacillus. I believe that there is danger in feeding butter to young children, and cannot see why those who demand milk from tuberculin-tested cows do not make the same demand for the butter for the children. It should also be made possible to purchase a certified or a pasteurized butter; that is to say, a safe butter. It is not necessary for me to pursue this subject further. I wish merely to call attention to the matter, at the same time adding that the idea is not quixotic, but has been carried out in Denmark and other countries for many years. The dealers in certified milk frequently have some of their produce left unsold, which with profit could be made into small prints of "certified butter." This subject seems to me to be timely for the consideration of the certified milk committees which have been so efficient throughout the United States.

SUMMARY

Of eighteen children who drank milk in which tubercle bacilli were demonstrated, all but one remained free from active tuberculosis during a period of supervision of three years; this one developed tuberculosis of the cervical glands, from which a bacillus of the bovine type was cultivated.

From animal experiments we can conclude that the chance of infection through milk depends not only on the number of bacilli ingested, but also on the frequency of exposure to small infections. For a quantity of bacilli which was insufficient to produce tuberculosis in guinea pigs when given in one dose, suspended in milk, was able to incite tuberculosis when fed in twenty-five to thirty doses on as many days.

Tubercle bacilli in milk are a menace to the health of young children. Tubercle bacilli in butter are likewise a menace; therefore, a safe butter, whether pasteurized or certified, should be provided for their use.

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CHOLERA

Charles Krumwiede, Jr., M. D., Josephine S. Pratt and Marie Grund, M. D.

Simple Methods of Bacteriological Diagnosis

In the examination of many specimens of fæces for the presence of cholera vibrios labor and time spent must be reduced to a minimum. This is especially evident in the examination of suspected carriers where it may be necessary to handle hundreds of cases a day. With such an amount of material even the best equipped laboratory would be crippled in attempting to carry out any but the simplest methods, especially in the face of an emergency. For reasons of expediency the following simple method has been adopted after trying different methods suggested by foreign workers:

Collection of material.—Fæces should be obtained whenever feasible and should reach the laboratory promptly, though if a large number of persons must be examined, the collection of fæces is almost impossible. The procedure adopted by Dr. Alvah Doty, Health Officer of the Port of New York, of taking rectal swabs is simpler and seems to be fairly reliable. These swabs are the ordinary wire, cotton-tipped swabs such as are used in diphtheria work. They should be moistened with sterile peptone solution to facilitate introduction and prevent drying during transit, which might cause the death of the vibrios if there is any delay in reaching the laboratory. We have distributed the swabs with several drops of peptone solution in the bottom of the tube, against which the cotton rests.

Media.—Three media have been used, *viz.*, Dunham's peptone water, Dieudonné's blood agar and alkaline 3 per cent. agar. Not all are necessary in routine work.

Outline of method—

Fæces.—Direct examination.

Peptone water.—Incubate 8 to 12 hours. Examine smears from surface growth. Examine those showing vibrios, in hanging drop, with and without agglutinating serum, making the hanging drops from the surface growth. If vibrios are too few in number for this, or if smears are negative, subinoculate into second peptone tubes.

Peptone water (subculture).—Incubate 8 to 12 hours. Examine as above. Those showing no vibrios or too few to see in the hanging drop are considered negative.

Direct examination.—This is of no value except in acute cases. If a large number of typical and extremely motile vibrios are present, the case may be considered positive. The difficulty is that occasionally stools from cases other than cholera show rather numerous vibrios.

Peptone water.—There is nothing to be gained in examining hanging drops from tubes in which no vibrios are revealed by smear. Further, it is a waste of time to examine the smear too long, for if the tubes contain cholera vibrios sufficient for diagnosis they are very quickly found. On the other hand, if they are few in number a second enrichment becomes necessary.

The surface growth in mixed cultures containing cholera is so nearly pure that on the addition of agglutinating serum the prompt clumping and loss of motility are sufficiently evident to be diagnostic. This application was suggested by Pollaci. It is seldom that other vibrios enrich to this extent. If they do, they can be excluded by the absence of any effect of the serum. When they are fewer in number, their motility may be like that of the cholera vibrio, which motility makes them evident even in hanging drops of mixed cultures. If the serum has no influence on this motility the vibrio can be excluded as not being cholera.

Subculture peptone water.—This is necessary, as in four instances we have found cholera vibrios in this way where the first series of tubes were negative. The fæces in these cases were not from cases showing clinical evidences of cholera. In the examination of acute cases no such necessity of further enrichment is likely to arise. In the examination of suspected carriers, however, an appreciable number of positive cases would probably be overlooked without a second enrichment. This second enrichment helps to exclude the vibrios other than cholera. Some will have died out; others will not have enriched beyond the amount present in the first tube; these are not cholera. All vibrios which have enriched are then differentiated with agglutinating serum.

In examining for carriers the examination of the first peptone tubes may be dispensed with altogether. No fear need be entertained that cholera vibrios will be lost; as a matter of fact, they will enrich still further in the second tube. This delays the findings in some instances, but becomes necessary where the number of examinations is large. In transferring the cultures the first peptone tubes should be inspected, as positive tubes show a characteristic surface cloud. Any tubes showing this should be examined at once.

This method requires no elaborate equipment and allows the examination of large numbers of cases with the least expenditure of time. If selective media are used, such as the Dieudonné or the modification described in the next section, they should be used in duplicate with the peptone tubes. The great advantage of this method is its applicability to emergency work.

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Selective Media: A New and Simple Modification

When Dieudonné prepared his alkaline blood agar he was the first to successfully apply a fact known for years, *viz.*, that the cholera vibrio could grow in the presence of alkali sufficient to inhibit other fæcal bacteria. Deycke had attempted the use of alkaline albuminates in 1893, but without uniform results.

The formula of Dieudonné's medium as now in use is as follows:

A. Defibrinated blood } a. a.
Normal sodium hydrate }

Mix thoroughly and steam in the Arnold sterilizer for three-quarters of an hour.

B. Meat peptone agar neutral to litmus containing 3 per cent. of agar.

Mix A. 30 parts and B. 70 parts while hot and pour thick plates. The plates are then left open for 20 minutes to dry. They are then placed in an oven at 50 to 60° for 20 to 30 minutes and finally left half open in the incubator over night. If used before this treatment cholera itself will not grow on the plates.

Since Dieudonné others have added facts which tend to simplify the preparations of the medium. Pergola found that the meat was not necessary in the agar, and that if this were left out the agar could be used without correcting the reaction. Recently Pilon has substituted a 12 per cent. crystalline sodium carbonate solution for the sodium hydrate. The plates can then be used at once; in fact, the blood mixture need not be heated if the plates are used immediately.

During our work with the Dieudonné medium it seemed to us susceptible of improvement in two particulars; first, if the medium could be rendered transparent; second, if a more easily obtainable material could be substituted for the blood. Where cholera examinations are of infre-

quent occurrence, however, or where blood is not easily obtained such a change would be extremely important.

Experiments were then undertaken, substituting eggs for the blood. Various mixtures were tried of the white alone, giving a transparent medium, and the whole egg, which gives a translucent medium. The following combinations were found satisfactory in that they showed the requisite amount of restraint of the fæcal bacteria with free growth of the cholera vibrio.

Egg white medium—

A. White of egg and water, a. a.
Sodium carbonate cryst. 12 per cent. } Mix in equal parts; steam in Arnold sterilizer for 20 minutes.

B. Meat peptone 3 per cent. agar, neutral to litmus.

Whole egg medium—

A. Whole egg and water, a. a.
Sodium carbonate cryst. 12 to 13½ per cent. } Mix in equal parts; steam for 20 minutes.

B. Meat-free agar, *viz.*, peptone, salt and 3 per cent. agar.

In either case mix A. 30 parts and B. 70 parts while the agar is boiling hot. Pour medium thick plates, allow them to stand open for 20 to 30 minutes to dry and then inoculate by surface streaking.

In making the egg mixtures the water and egg must be thoroughly mixed, the alkali added and again well shaken. Filtration through a thin layer of cotton may be employed to remove any of the thicker parts of the egg.

Of the two formulæ the whole egg gives the more vigorous growth of vibrios, requires no meat in the agar, and is more economical. The egg white, on the other hand, requires a meat agar, the growth is not as vigorous, and crystals frequently form, especially along the inoculation streaks. Its only advantage is its transparency. For general use, therefore, the whole egg mixture is better. Comparing the whole egg medium with the blood medium of Dieudonné, the latter is somewhat more selective, that is, it seems to restrain the common fæcal bacteria somewhat more. This, however, is more than offset by the fact that should other bacteria grow the cholera colonies on the egg medium are distinctive. Examined by transmitted light they have the appearance of being deep in the agar and have a peculiar hazy look, due to a halo about the colony. Where the growth is very vigorous this halo is surrounded by a zone of clearing. In practice fishing from this type of colony has yielded cholera and from this type alone. The only exception is in the case of cholera-like vibrios, their colonies being identical. The plates only keep, when stored on ice, for a limited time. The egg alkali mixture seems less permanent. This is unimportant compared with the more permanent Dieudonné blood mixture, as the egg mixture can be prepared so quickly.

Because of the ease of preparation and the prompt availability of the ingredients, this medium is an improvement, especially for those who are called upon to examine cholera-suspected stools at infrequent intervals. When one considers that ten eggs is sufficient for over 300 plates the medium is economical, even where large numbers of examinations are being done. Furthermore, it is an improvement for the reason that cholera grows in distinctive colonies, allowing quick selection for smears and agglutination.

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Cholera-like Vibrios

The vibrios other than cholera found in stools are of little practical importance. When, however, the present trouble arose there was no cholera-agglutinating serum available for immediate use. Under these circumstances the detention of a ship was necessary because of the presence of a cholera-like vibrio in one case. This is an emergency that is likely to arise at any time in a country where cholera examinations have not been required for many years.

Because of this we have tested the various vibrio strains we have isolated to see if there were any biological peculiarities which would quickly exclude those not cholera. This has yielded some results. Some of the vibrios can be excluded because of the marked tenacious pellicle they form on peptone or the dry, adherent growth on agar. Of fifty vibrios other than cholera, forty-three did not give the cholera-red reaction.* Of the seven that did, two did not produce acid and four produced gas; in litmus-glucose peptone only one produced acid-like cholera, but this strain formed a tough pellicle and was adherent on agar. These differences were evident in less than 24 hours' incubation.

If, then, a colony be fished and inoculated into peptone water, glucose media and agar, many, if not the majority, of vibrios other than cholera could be quickly excluded.

The ability to liquefy Löffler's blood serum and gelatine and the fermentation of saccharose and lactose as well as milk were tried. The acid change in lactose, milk, and the liquefaction of gelatine and Löffler, as exhibited by cholera, is too slow to be of practical use. The fermentation of saccharose is more prompt. About half of the other vibrios ferment this sugar, some with the production of gas.

A few vibrios we have found to be only slightly motile. When mixed cultures containing vibrios are sub-inoculated in peptone water they do not show the same ability to enrich as shown by cholera. Many enrich to a limited degree and then remain constant. Others enrich slightly and die out in the third tube of peptone. This may be considered evidence that cholera is not present.

Hæmolytic Action of Cholera and Cholera-like Vibrios

Because of the number of varied reports that have appeared on the subject of hæmolysis by cholera and other vibrios, we have tested fifty cholera and fifty non-cholera strains for their hæmolytic properties. These were recently isolated and included one culture from the Philippines and two from Italy. One old culture isolated by Buxton in 1893 and one El Tor strain were also tested. This latter strain is one of the six isolated in 1905 by Gottshlich at El Tor from the cadavers of pilgrims to Mecca. These pilgrims had died with diarrhœal symptoms, but had shown no clinical evidences of cholera. The El Tor strains agglutinated like true cholera, but were strongly hæmolytic. The hæmolytic power was considered by some, especially Kraus and Ruffer, to be sufficient evidence for regarding these vibrios as not true cholera. This was based on Kraus' statement that the hæmolytic power was not a property of true cholera, but was only found in allied types.

* Care should be used in determining this, as in several instances we got a reaction with peptone water to which sodium hydrate had been added to increase the alkalinity, not otherwise. Whether this was due to traces of nitrite we cannot say.

Our results were as follows:

Thirteen non-cholera vibrios	weakly hæmolytic.
Four non-cholera vibrios	strongly hæmolytic.
El Tor	strongly hæmolytic.
Cholera, Buxton*	strongly hæmolytic.

The following technic was employed: To 1 c. c. of a suspension of washed sheep, goat, or horse corpuscles was added ¼ c. c. of a week-old broth culture.

Readings were made after two and three hours' incubation. The tubes were then shaken and put on ice for 24 hours, when final readings were taken.

Our results with the cholera vibrios agree with those of Mühlens, Von Raven, Neufeld, and Hændel and others who have found that laboratory cultures of true cholera may possess or acquire hæmolytic power.

On the other hand, Mühlens and Von Raven found that three rather recently isolated strains possessed hæmolytic powers. Bærthlein also found some newly isolated strains strongly or weakly hæmolytic, depending upon the technic used. When he inoculated corpuscle suspensions from agar slants and incubated 24 hours or longer he found one strain strongly and others irregularly hæmolytic. When he used a five-day bouillon culture the same strain showed comparatively prompt hæmolysis. All, however, showed varying degrees of hæmolysis at 48 to 72 hours. With blood agar plates again only the one strain showed hæmolysed areas.

Although the technic of different observers has differed so greatly as to make it difficult to correlate the results, there can be no doubt that there is great variability in the hæmolytic powers on successive tests even of the El Tor cultures. Freshly isolated cholera cultures do not possess strong hæmolytic powers, though this property may be acquired after artificial cultivation for longer or shorter periods of time. In a few cases freshly isolated strains have been strongly hæmolytic or have shown traces of this capacity. This capacity may not be as well developed as in some non-cholera vibrios, but it cannot be considered of sufficient importance to justify the separation of strains biologically true cholera, but hæmolytic, from non-hæmolytic strains. If a strain, El Tor or other vibrio, agglutinates in a cholera-immune serum, it must be considered cholera, whether it hæmolyses or not. The fact that the El Tor strains were isolated from pilgrims who must have become infected months previous to their death, that is, were carriers for an unusually long period, and who, during the pilgrimage, were not the source of cases of cholera, is none the less remarkable than that each strain isolated should be the same in possessing hæmolytic properties.

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Kraus, R., Graham, A. L., and Zeky Zia. Über Hämotoxine und die Blutplatten Methode. Deut. med. Woch., 1911, p. 1471.

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* This strain showed cultural variations. It did not liquefy Löffler's blood serum and was extremely slow to liquefy gelatine. We could not test its agglutinability as it made a poor suspension, which quickly flaked out. In rabbits, however, it produced agglutins for true cholera.

THE VIABILITY OF THE BACILLUS MALLEI IN WATER

M. Grund, M. D.

In connection with the question of the common drinking trough as a factor in spreading glanders, a number of tests were made to determine the viability of the bacillus mallei in water. Two strains of the organisms were used, one of which had recently been isolated from a glandered horse, while the other was an old laboratory stock culture which had not been passed through an animal for at least two years. They were grown in ordinary nutrient broth for 24 hours, and were diluted before plating with sterile distilled water, or with a sterilized mixture of sputum and water. To eliminate all nutrient material, an emulsion of a 24-hour agar culture in normal salt solution, of the same density as the broth culture, was used in a parallel series of tests. The dilutions were plated on glycerine potato agar (a) at once, (b) after two hours, (c) after 24 hours at room temperature, and all plates incubated 48 hours. At the end of that time the plates marked (a) and (b) showed many colonies, while on those marked (c) relatively few organisms seemed to remain alive and to multiply.

Further tests were then made by keeping the broth culture and the salt suspension diluted with sterile water at room temperature for a number of days. The dilutions of broth culture showed more colonies than those made of the salt suspension, but even in the latter a small number of colonies developed after 28 days, showing that the bacillus mallei may remain alive in distilled water, without the addition of any nutrient material, for nearly a month. The recently isolated culture showed greater viability than the old stock culture.

A NOTE ON THE SEARCH FOR THE CAUSATIVE FACTOR OF SCARLET FEVER: A BACTERIOLOGICAL STUDY

(A Discussion of Dr. Kolmer's Paper at the Academy of Medicine)

Matthias Nicoll, Jr., M. D.

In July last I undertook a study of the enlarged lymph nodes occurring in scarlet fever, according to the method of Dr. Vipond,† and as recently done by Dr. Kolmer.‡ With the usual precautions as to disinfection of the skin, etc., I aspirated into a syringe, which had been thoroughly boiled, some of the contents of an enlarged cervical node in two cases of scarlet fever, inoculated various media and produced no growth. Owing to lack of material, the study was not resumed until October, when another fresh case gave opportunity for the same procedure. What was my surprise to obtain on all the inoculated tubes a pure culture of a spore-bearing bacillus. On the following day another node in the same case gave an identical result. Five cases successively produced the same bacillus from inguinal nodes. Owing to the presence of this organism in the original smears, I was able to overcome the scientific scepticism of the director, Dr. William H. Park, and, to some extent, the hatred of a spore-bearer among his staff of bacteriologists, and an exhaustive study of the cultural characteristics and morphology of this organism was made by Drs. Krumwiede, Grund and others. Guinea pigs, rabbits and one monkey were inoculated with 24-hour broth cultures, and in all were produced fever, marked diarrhœa, loss of flesh and moderate enlargement of the neighboring lymphatics, from which also the bacillus could be recovered after a week. No rash was observed in any case; neither was there desquamation. At this time Dr. Vipond, at my suggestion, brought to us his organisms for comparison, and it was soon discovered that there was between them marked morphologic and cultural differences. It furthermore developed that our organism was extremely resistant to heat, surviving boiling under pressure for at least 20 minutes. The syringes were then autoclaved on two successive days, and the experiment repeated on eleven other patients, on some more than once, with the result that no organism of any kind was found. I may add that Dr. Vipond personally performed the aspiration on two of these patients. Furthermore, the organism which he brought with him was inoculated into four rabbits and two monkeys and produced practically the same symptoms as that which we had isolated. Where did the latter come from? The piston of the syringes which I had used in the work and which had subsequently been boiled was stirred into broth and plates inoculated. From all our bacillus was obtained. Furthermore, six stock syringes, which had never been used, gave the same result. This work, I believe, was not without value, since it has taught us the necessity of absolutely indisputable sterilization in all such experiments, and it has demonstrated what seems to me a most important fact, namely, that in 18 cases of scarlet fever, ill from 2 to 24 days, enlarged, but not suppurating, lymphatic nodes, in 4 cases in the cervical region and in 19 in the inguinal region, together with an inguinal node removed post-mortem from a septic case of the disease, in no instance showed the presence of streptococcus or other organism. Direct smears from all but the first two cases showed by the presence of lymph cells that the node had been tapped. Here, then, is a disease which involves the lymphatic system from the neck to the groin in 48 hours or less, and does so apparently without the aid of pyogenic organisms. Dr. Kolmer's series of 11 cases, recently published, justifies the same conclusion. Before closing I wish to express my deep-

† *Archives of Pediatrics*, 1911, p. 564.
‡ *American Journal of Diseases of Children*, Vol. II., No. 5, p. 329.

rooted suspicion of all reported cases of experimental scarlet fever occurring in rabbits, such, for instance, as Cantacuzene has recently announced. The rash of scarlet fever is frequently difficult of diagnosis in human beings and such a rash in an unshaved rabbit must necessarily be largely a matter of personal opinion. On the other hand, the shaving of normal rabbits, owing to the delicacy of their skin, produces a dermatitis, persisting for a number of days, which an enthusiast might reasonably ascribe to scarlet fever. Furthermore, the temperature curve in these animals is so erratic and readily disturbed that little is to be deduced from this source, while their throats and tongues are very unsuited to clinical observation.

THE PREPARATION OF DIPHTHERIA AND TETANUS ANTITOXIC PLASMA FOR THERAPEUTIC USE

EDWIN J. BANZHAF, PHAR. D.

It might be recalled that during the year 1908 a method was devised in this laboratory for the further purification of diphtheria and tetanus antitoxins. The essential part of this method consisted in heating the native antitoxic plasma for 15 hours at 56° Centigrade.

Further studies in purification of these antitoxins have been continued. We have found that a still further separation and change in the precipitation characterization of the non-antitoxic pseudo-globulin can be obtained. This is accomplished by diluting the native antitoxic plasma with one-third its volume with water and adding sufficient sodium chloride so that the diluted plasma contains 1½ per cent. After this treatment the diluted plasma can be heated for 15 hours at 56° Centigrade or for 6 to 7 hours at 57° Centigrade. The technic of the process of purification has been improved, especially in the pulp filtrations; this and one other heretofore insurmountable condition which entailed a considerable loss of antitoxin has also been overcome. We refer to the frequent clouding of the sterile antitoxic globulin solution, which was found to be due to the chloroform that had been added as a preservative.

On removing the antitoxic globulin solution from the dialyzing bag, we now add slowly 10 per cent. of a 4 per cent. emulsion of trikresol to the antitoxic globulin solution. While the trikresol emulsion is being added the antitoxic solution should be stirred rapidly. No flocculation or coagulation of protein takes place.

The antitoxic globulin solution containing the trikresol is placed in the ice chest for at least two weeks. It is then filtered through a very thin layer of paper pulp, covered with fine sand and then through a sterile Berkefeld. Treated as above, the antitoxic globulin solution will remain clear for an indefinite period.

VAUGHAN'S SPLIT PRODUCTS AND UNBROKEN PROTEINS: A COMPARATIVE STUDY OF THEIR EFFECTS

EDWIN J. BANZHAF, PHAR. D., AND EDNA STEINHARDT, M. D.

As is well known, Vaughan[1] and his students have taken proteins, rendered fat-free by extraction with alcohol and ether, and treated them at a temperature of 78° C., with absolute alcohol, in which 2 per cent. sodium hydroxide had been dissolved. The protein, thus treated, breaks into a poisonous and non-poisonous part, the former soluble in the menstruum, the latter insoluble. These constitute Vaughan's split products.

The poison is soluble in the sodium hydroxide alcohol. It is non-specific, and has the same physiological action and a closely allied chemical composition in all proteins examined. It kills normal guinea pigs when injected intraperitoneally, with symptoms identical with those produced in serum anaphylaxis.

The insoluble portion is designated by Vaughan as the residue. This is specific for each protein. With it guinea pigs can be sensitized, as well as with the unbroken protein. According to Vaughan's theory, in the case of bacteria, this sensitization results in the destruction or splitting of bacteria, with the liberation of the poison. This gives immunity if the quantity of the bacteria is not too great; if too great, death from poisoning occurs. In the case of dead proteins, as serum or egg-white, there is a greater amount of protein injected, and a greater amount of poison is set free by the splitting, producing toxic symptoms. The result here is anaphylaxis, the process being the same in bacterial immunity and protein hypersensitization.

To quote Vaughan and Wheeler[2]:

"Protein susceptibility and immunity are different manifestations of one and the same process. Both depend upon the development in the animal body of a specific ferment. When the specific ferment splits up a living protein before it has time to multiply, we say that the animal is immune. When this cleavage action is less prompt, but sufficiently so to split up the living protein before it elaborates a fatal amount of the poison, the animal sickens but recovers. When this action is still less prompt and the living protein constructs enough poison to kill, then its liberation causes death. When this specific proteolytic ferment has been developed in the animal by previous treatment with a dead or stable protein, it is easy to inject a small dose of the same protein in sufficient quantity to quickly induce symptoms and to kill. Then we say that the animal has been sensitized, or is in a condition of hypersensibility."

And again Vaughan,[3] discussing immunity and hypersensibility, says:

"The apparent difference arises from the fact that in one instance we are dealing with a dead stable protein, and in the other with a living labile protein."

Dr. Vaughan kindly sent us some of his split products of egg-white. The effects of the poison and the residue were compared with those of unbroken horse serum and irrespective of unbroken egg-white, the anaphylactic phenomenon being almost identical.

The work falls under the following heads:

THE POISON

1. Comparative inoculations.
2. Effect of heat.
3. Effect of chloral.
4. Effect of lecithin { On serum anaphylaxis. / On the poison.

THE RESIDUE

1. Sensitizing action of various samples.
2. Absence of vaccinating action.

EFFECTS OF INOCULATION

The poison.—The poison in aqueous solution is acid. With the unneutralized solution, intraperitoneal or intracerebral injections produced typical symptoms of anaphylaxis, resulting in death.

When the aqueous solution is made neutral by the addition of sodium bicarbonate a precipitate is formed. On filtering, according to Vaughan,[1] the filtrate contains the poison. Upon using neutralized solutions the action was much weakened. Intraperitoneally, even 100 mm., although giving grave symptoms, failed to kill. Intracerebrally, even in saturated solutions, with the sample of poison we had, the symptoms were slower in coming on, not as explosive as in serum anaphylaxis, and death, if it occurred, was greatly retarded (after half an hour).

Intracardiac injections of 15 to 20 mm. in neutralized solutions killed in one to two minutes, giving the same symptoms as in serum anaphylaxis. As intracardiac injections with neutralized solutions of this sample of the poison gave the most typical results, we were forced to adopt that method for all of the experiments done with it. Realizing, however, that intracardiac injections very frequently result in injury to the heart muscle, or lungs, with resulting hemorrhages, and also the possibility of the whole dose not entering the heart, experiments were repeated many times to make our results positive. Autopsies were performed in all cases in which death occurred, to exclude mechanical injury with resulting hemorrhages or shock as its cause.

In some of the later experiments a second specimen of poison sent us through the kindness of Dr. Vaughan was used. This, when injected in unneutralized solutions intraperitoneally in 13.7 mm. doses, gave typical symptoms and death in from 5 to 20 minutes.

Effects of heat.—Normal horse serum, rendered slightly alkaline by the addition of sodium bicarbonate and diluted, was heated to 100° C. for 15 minutes. Intracardiac injections into a sensitized guinea pig gave no symptoms. One hour later an injection of unheated serum was given and death resulted with typical symptoms. This is in accord with the results of Dœrr and Russ[4] and others, who find that heating serum from 96° to 100° C. destroys its toxic action.

We believe this loss of toxic action to be due to a partial coagulation of the proteins, thereby retarding the action of the active agent in the sensitized guinea pig.

The poison when heated to 100° C. for 15 minutes showed no lessening of its action. However, Besredka[5] found that milk could be heated to 120° C. without destroying its toxic properties for sensitized guinea pigs.

Through the kindness of Dr. Vaughan one of us was permitted to test further in his laboratory the heat resistance of his poison. A poison split from egg-yolk was used of which one cubic centimeter unneutralized injected intraperitoneally in guinea pigs was the M.L.D. Death resulted in 5 to 20 minutes. When heated to 100° C. for 15 minutes it still killed promptly in one cubic centimeter doses. Autoclaved at 120° C. for 15 minutes, the solution, which before heating was clear, became cloudy. One cubic centimeter gave retarded and prolonged symptoms followed by recovery. Three cubic centimeters killed with typical symptoms in 10 minutes. Therefore, 120° C. for 15 minutes partially destroys the poison, possibly through partial coagulation.

Effect of Chloral.—Solutions of chloral hydrate were given intracardially according to the methods of Banzhaf and Famulener.[6] The guinea pigs were given divided doses of the chloral intracardially until complete hypnosis was produced, then a lethal dose of the poison was injected. Guinea pigs treated in this manner are protected against a lethal dose of

TABLE I

INOCULATIONS

Weight	Site of Inoculation	Poison Un-neutralized mgr.	Poison Neutralized and Filtered mgr.	Symptoms	Result	Autopsy
220	Intracerebral	5		Typical*	Death 2 to 3 minutes	No cerebral hemorrhage.
180	Intracerebral		5	15 to 20 minutes after inoculation, symptoms, then convulsions lasting 15 minutes	Death in 35 minutes	No cerebral hemorrhage.
225	Intracerebral		5	3 minutes after inoculation, excitement; 10 minutes later convulsive movements, lasting 30 minutes	Death in 45 minutes	No cerebral hemorrhage.
300	Intraperitoneal	100		Typical	Death in 15 minutes.	
300	Intraperitoneal		100	Typical; grave	Recovered in one-half hour.	
210	Intracardiac		20	Typical	Death in 2 minutes	No hemorrhage in pericardium.
280	Intracardiac		15	Typical	Death in 2 minutes	No hemorrhage in pericardium.
215	Intracardiac		10	Severe symptoms for 5 to 6 minutes	Complete recovery in 1 hour.	

*Symptoms similar to those of anaphylaxis.

TABLE II

EFFECT OF HEAT ON VAUGHAN'S EGG-WHITE POISON (NEUTRAL SOLUTION) AND ON HORSE SERUM

Guinea-pig	Previous Treatment	Amount Injected	Site of Inoculation	Symptoms	Result	Autopsy
Normal	.....	Poison, 15 mg.*	Intracardiac	Typical	Death within 2 minutes	No evidence of hemorrhage in pericardium
Normal	100° C. for 15 minutes	Poison, 15 mg.*	Intracardiac	Typical	Death within 2 minutes	No evidence of hemorrhage in pericardium.
Sensitized to horse serum	100° C. for 15 minutes	1 c. c. horse serum diluted	Intracardiac	None.		
Same guinea-pig	.....	1 hour later 1 c. c. horse serum	Intracardiac	Typical	Death within 2 minutes	No evidence of hemorrhage in pericardium.

*Each cubic centimeter of the solution of the egg-white poison contained twenty milligrams of the poison dissolved in sterile water. It was neutralized with sodium bicarbonate and filtered. Of this filtrate three-fourths cubic centimeter equal to fifteen milligrams of the poison was the M.L.D.

the poison. If two or more lethal doses are given the animal dies within two minutes with typical symptoms.

Mixture of Chloral and the Poison in Vitro.—The required amount of chloral was mixed with a lethal dose of the poison and allowed to stand at room temperature from 10 minutes to half an hour. The mixture was then given intracardially to a guinea pig. The results were variable. Occasionally the animal would immediately go under the effects of the chloral, become unconscious and might show a few twitchings, and awaken at the end of one hour or more well or, in other words, protected.

Again, there were convulsions and death from the poison within two minutes; or, again, partial protection and partial hypnosis with delayed poison symptoms, usually resulting in death.

These irregular results we interpreted as meaning that no chemical combination had taken place in vitro, and that it depended on which molecules in sufficient quantity in the mixture reached the vital cells first, whether protection or death occurred, and that if part of each reached these cells, partial protection occurred. Evidently the protection is due either to a combination of the chloral with the nerve cells, preventing the entrance of the poison, which is then eliminated or taken up by non-vital cells; or the chloral and some constituent of the vital cells form a combination, which, while it may not prevent the entrance of the poison into the cell, prevents its shock from being transmitted.

TABLE III

Chloral Hypnosis Followed by Injections of Vaughan's Egg-White Poison

G. P. Weight	Chloral Hydrate Intracardially	Poison Intracardially	Symptoms	Results	Autopsy
210.....	30 mg. 30 "	20 mg.	No change in respiration.	Recovery from chloral complete after 3 hours. No poison symptoms.	
215.....	30 " 30 "	20 "	No change in respiration.	Recovery from chloral complete after 3 hours. No poison symptoms.	
375.....	50 " 50 "	20 "	No change in respiration.	Recovery from chloral complete after 3 hours. No poison symptoms.	
275.....	.....	20 "	Typical.	Death within 2 minutes.	No evidence of hemorrhage in pericardium.

Solutions of poison used as in Table No. 2: 1 c. c. = 20 mg. (M.L.D. = 15 mg.)

The dilutions for injection were such that not more than two cubic centimeters were given. As this was injected slowly, the danger from possible dilatation of the heart could be largely ruled out. Many more animals were tested, but those are sufficient to show the irregularity.

TABLE IV

Mixture of Chloral and Poison in Vitro

G. P. Weight	Chloral Hydrate Poison Intracardiaily	Mixture Stood at Room Temperature	Symptoms	Result	Autopsy
240.....	65 mg. chloral. 20 " poison.	30 minutes.	Slow effect of chloral; cough; slight convulsions then chloral hypnosis.	After 5 hours animal still unconscious; found dead next A.M.	Hemorrhage in heart.
280.....	85 " chloral. 20 " poison.	10 "	Completely under chloral; no symptoms.	After 1 hour recovered.	
260.....	75 " chloral. 20 " poison.	10 "	Typical.	Dead within 1 minute.	No hemorrhage in pericardium.
275.....	70 " chloral. 20 " poison.	10 "	Completely under chloral; no symptoms.	After 1 to 2 hours recovered.	
250.....	65 " chloral. 20 " poison.	10 "	Typical.	Dead within 2 minutes.	No hemorrhage.
280.....	85 " chloral. 20 " poison.	30 " "	"	Dead within 2 minutes.	No hemorrhage.
295.....	90 " chloral. 20 " poison.	30 " "	"	Dead within 3 minutes.	No hemorrhage.

Through the kindness of Dr. Vaughan we obtained another sample of poison from egg-yolk. This, in unneutralized solution, was fatal to guinea pigs in from 5 to 15 minutes, in intraperitoneal doses of one cubic centimeter. Each cubic centimeter of this solution contained 13.7 milligrams of the poison.

TABLE V

Intramuscular Injections of Chloral Hydrate Followed by One Cubic Centimeter of Vaughan's Egg-Yolk Poison Intraperitoneally

G. P. Weight	Chloral Hydrate Intramuscularly	Poison Intraperitoneally	Symptoms	Results
265.....	90 mg.	1 c. c.	None.	Recovered.
295.....	110 "	1 "	"	"
280.....	100 "	1 "	"	"
300.....	115 "	1 "	"	"
275.....	95 "	1 "	"	"
280.....	100 "	1 "	"	"
290.....	.....	1 "	Typical	Death within 10 minutes.
300.....	.....	1 "	"	" " 15 "

Solutions of chloral hydrate were given intramuscularly according to the method of Banzhaf and Famulener.[6] When the desired degree of hypnosis was produced one cubic centimeter of the egg-yolk poison was given intraperitoneally. Guinea pigs treated in this manner were completely protected from the effects of the poison. If more than one fatal dose of the poison was given the animal died within 10 to 20 minutes with typical symptoms of anaphylaxis.

Lecithin.—Following the idea that the toxic effect in anaphylaxis and in the poison was due to a combination with some of the cell constituents, the effect of lecithin was studied. An egg lecithin (Kahlbaum) was first used, later an egg lecithin (Merck's) was used, both giving like results.

De Waele[8] found that, while small doses of lecithin increased, larger doses decreased the effects of alkaloids. We, therefore, used both small and large doses.

Lecithin—Serum Anaphylaxis.—Lecithin in doses from 25 to 750 milligrams was emulsified very thoroughly with five cubic centimeters of serum, allowed to stand at room temperature for one hour, and then injected intraperitoneally into sensitized guinea pigs. At the same time normal guinea pigs were injected with duplicate doses. The inoculations in both instances where the large doses of lecithin were used caused at once symptoms of peritoneal irritation (solutions were slightly acid), then weakness of the hind legs, rapid breathing, and possibly partial collapse. In the normal guinea pigs recovery was gradual, leaving a condition of malaise at the end of about 45 minutes.

The sensitized animals showed typical anaphylaxis. There was no protection. Repetition of these experiments gave the same results.

Lecithin Followed by Serum.—Five hundred milligrams of lecithin emulsified thoroughly in five cubic centimeters of sterile water were injected intraperitoneally into sensitized and normal guinea pigs.

In both series of animals the immediate irritation effects occurred, followed by weakness of hind legs and partial collapse, as in the former experiments.

TABLE VI

Small Doses of Lecithin and Serum Contact One Hour

G. P. No.		Interval in Days	Second Injection Intraperitoneally	Symptoms	Results
784.....	.75 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	25 mg. lecithin + 3 c. c. horse serum.	Typical anaphylaxis.	Death within 15 minutes.
781.....	.75 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	25 mg. lecithin + 3 c. c. horse serum.	Typical anaphylaxis, very grave.	Gradual recovery.
708.....	.75 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	3 c. c. horse serum.	Symptoms as No. 781.	Gradual recovery.
318.....	Normal	.....	25 mg. lecithin + 3 c. c. horse serum.	Slight abdominal irritation.	Rapid recovery.
320.....	"	.....	25 mg. lecithin + 3 c. c. horse serum.	Slight abdominal irritation.	Rapid recovery.

TABLE VII

Large Doses of Lecithin and Serum (In Contact One Hour)

G. P. No.		Interval in Days	Second Injection Intraperitoneally	Symptoms	Results
794.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	500 mg. lecithin + 5 c. c. horse serum.	Abdominal irritation; then typical anaphylaxis.	Death within 15 minutes.
754.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	500 mg. lecithin + 5 c. c. horse serum.	Abdominal irritation; then typical anaphylaxis.	Death within 20 minutes.
751.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	750 mg. lecithin + 5 c. c. horse serum.	Abdominal irritation; then typical anaphylaxis.	Death within 20 minutes.
721.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	5 c. c. horse serum.	Typical anaphylaxis.	Death within 20 minutes.
720.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	5 c. c. horse serum.	Typical anaphylaxis.	Death within 20 minutes.
317.....	Normal.	.....	500 mg. lecithin + 5 c. c. horse serum.	Abdominal irritation; weakness hind legs; partial collapse.	After 45 minutes recovery save for malaise.
322.....	"	.....	750 mg. lecithin + 5 c. c. horse serum.	Abdominal irritation; weakness hind legs; partial collapse.	After 45 minutes recovery save for malaise.

Nineteen hours later all the animals had recovered. Five cubic centimeters of normal horse serum were then injected intraperitoneally. The normal guinea pigs were injected intraperitoneally. The normal guinea pigs showed no symptoms. Of three sensitized animals two showed no symptoms, the third scratching at nose and body. This animal showed no further symptoms of anaphylaxis. Six hours later an additional five cubic centimeters of normal horse serum were injected intraperitoneally; no symptoms followed. Repetitions of the above experiments gave similar results. Here, however, the second five cubic centimeter intraperitoneal injection was given 24 hours after the first five cubic centimeters, and in some instances this caused slight but distinct symptoms, scratching at nose and biting at fur at site of injection. These symptoms in some instances lasted half an hour.

TABLE VIII

Lecithin, Five Hundred Milligrams Intraperitoneally, Followed in Nineteen to Twenty-Four Hours with Five Cubic Centimeters Normal Horse Serum

G.P. No.	Previous Treatment	Interval in Days	Lecithin Emulsion	5 c.c. Horse Serum Intraperitoneally	Symptoms	Results
862.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	500 mg.	19 hours later.	Scratching at nose.	Recovered.
751.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	28	500 "	19 hours later.	None.	"
779.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	500 "	24 hours later.	"	"
740.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	500 "	24 hours later.	Scratching at nose.	"
766.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	500 "	24 hours later.	None.	"
789.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	28	Control.	24 hours later.	Typical.	Death within 20 minutes.
781.....	.73 c. c. toxin + 1/400 c. c. antitoxic horse serum.	29	"	24 hours later.	"	Death within 15 minutes.

Measured amounts of emulsified lecithin were given intraperitoneally to determine the smallest amount that would protect sensitized guinea pigs for the second injection of horse serum.

Four sensitized guinea pigs received 10 mg. lecithin.
" " " " " 25 " "
" " " " " 50 " "
" " " " " 100 " "
" " " " " 250 " "

Twenty-four hours later all of these animals received five cubic centimeters normal horse serum intraperitoneally. The four that received 10 milligrams and two that received 25 milligrams of lecithin developed typical symptoms, dying within 40 minutes; the other two had very severe symptoms lasting about 45 minutes, followed by recovery.

The four that received 100 milligrams of lecithin had severe symptoms lasting about 20 minutes, followed by recovery. Those that received 250 milligrams of lecithin had slight but definite symptoms of anaphylaxis.

Lecithin and the Poison.—Injections of various amounts of lecithin and the poison in normal guinea pigs. Emulsion of lecithin and the poison was given after standing in contact one-half hour; also emulsion of lecithin was given, followed after 24 hours with the poison. Neither small nor large doses of the lecithin, 50 to 500 milligrams, mixed with one fatal dose of the poison in vitro had any neutralizing effect.

TABLE IX

EMULSION OF LECITHIN AND EGG-POISON (IN CONTACT ONE-HALF HOUR)

G. P. No.	Intraperitoneal Injection	Symptoms	Results
250.....	50 mg. lecithin + 1 c. c. poison.	Typical.	Death within 20 minutes.
251.....	500 " " " 1 " "	"	" " 24 "
252.....	1 c. c. poison.	"	" " 15 "

TABLE X

LECITHIN FIVE HUNDRED MILLIGRAMS INTRAPERITONEALLY, FOLLOWED IN TWENTY-FOUR HOURS BY EGG-POISON

G. P. No.	Intraperitoneal Injection of Lecithin	Intraperitoneal Injection 24 Hours Later	Symptoms	Results
210.....	500 mg.	1 c. c. egg poison.	Typical.	Death within 15 minutes.
211.....	500 "	1 " " "	"	" " 15 "
212.....	500 "	1 " " "	"	" " 15 "

THE RESIDUE

The Non-poisonous Portion.—Vaughan and Wheeler[2] in their work on egg-white and its split products showed that guinea pigs are sensitized to a second injection of egg-white by a small preliminary dose, exactly as in serum anaphylaxis. This sensitization to whole egg can be effected by the residue as well as by the unbroken protein. The residue does not sensitize to itself, and no anaphylactic symptoms are produced by even large doses of the residue in a sensitized guinea pig.

This they consider evidence of a complete separation of the poisonous and non-poisonous portions.

Dr. Vaughan very kindly sent us a sample of egg residue. On testing it we found to our surprise that it had no sensitizing action. More residue was then sent to us from another lot made at a different date, but this also was inactive.

Recently Dr. Vaughan has permitted one of us to investigate the occasional non-sensitization of his residue in his own laboratory (Hygienic Laboratory, University of Michigan).

After testing three residues one was found which had a sensitizing action, the other two being inactive.

The process of preparing the residue is as follows:

The residue after being coagulated in 96 per cent. alcohol is extracted with alcohol and ether. After drying it is treated with absolute alcohol containing 2 per cent. sodium hydroxide for one hour at 78° C. (the boiling point of absolute alcohol); when cooled this menstruum is poured

TABLE XI

EGG-WHITE RESIDUE NOS. 1 AND 2 (VAUGHAN) NO SENSITIZATION

G. P. No.	Residue No. 1	Interval in Days	Intracerebrally Equal Amounts Egg-white + .8% Salt Solution	Symptoms	Result
1.....	50 mg. subcutaneously.	24	¼ c. c.	None.	
2.....	50 mg. subcutaneously.	24	¼ "	"	
3.....	50 mg. subcutaneously.	24	¼ "	"	
4.....	50 mg. intraperitoneally.	24	¼ "	"	
5.....	50 mg. intraperitoneally.	24	¼ "	"	
6.....	50 mg. intraperitoneally.	24	¼ "	"	
7.....	Egg-white + .8% salt solution.	24	¼ "	Typical.	Death within 6 minutes.
8.....	1 c. c. subcutaneously.	24	¼ "	"	Death within 5 minutes.
	Residue No. 2				
410.....	50 mg. subcutaneously.	15	Intraperitoneally equal amounts.	None.	
411.....	50 mg. subcutaneously.	15	Egg-white + .8% salt solution 5 c. c.	"	
412.....	25 mg. subcutaneously.	15	Egg-white + .8% salt solution 5 c. c.	"	
413.....	25 mg. subcutaneously.	15	Egg-white + .8% salt solution 5 c. c.	"	
414.....	Egg-white + .8% salt solution 1 c. c. subcutaneously.	15	Egg-white + .8% salt solution 5 c. c.	Typical.	Death within 30 minutes.
415.....	Egg-white + .8% salt solution 1 c. c. subcutaneously.	15	Egg-white + .8% salt solution 5 c. c.	"	Death within 30 minutes.

off and the residue again treated with the fresh alcohol and sodium hydroxide for one hour at 78° C. This treatment is repeated a third time. Thus the residue, or insoluble part of the egg-white, has been submitted to a temperature of at least 78° C. for three hours.

Recently, however, the residue has been heated on a compressed steam bath with a flask set rather deeply in the bath. The bottom of the flask is probably heated to 98° or 99° C., and, as the flask is not shaken, that part of the egg-white which is in close contact with the glass may have been heated considerably over the supernatant alcohol.

Although investigators have found varying temperatures to cause the disappearance of the sensitizing, vaccinating and intoxicating properties of unbroken proteins (especially horse serum) as shown by Besredka in his recent article, shorter periods than three hours have always been tried for the heat treatment.

Doerr and Russ[4] have found that it is occasionally impossible to sensitize with horse serum heated to 80° C.

If sufficient of the residue was in contact with the bottom of the flask, the temperature of that part of the residue might be raised over the supernatant alcohol boiling at 78° C., and this raising of the temperature might cause the inactivity. For by submitting whole proteins (horse serum) to gradually increasing temperatures the toxic and vaccinating properties are first lost; the sensitizing power disappears later. Therefore, irrespective of any splitting action of the alcohol and sodium hydroxide on the proteins, the prolonged heating alone might account for most, if not all, of the modification which the residue shows. For Vaughan and Wheeler[2] have already shown that their residue has neither toxic nor vaccinating properties.

The finding of inactive residue now completes the parallel of the effect of heat on the unbroken protein (horse serum).

Non-production of Anti-anaphylaxis with the Residue.—Vaughan and Wheeler[2] show that the residue, while it sensitizes to the unbroken protein, does not sensitize to itself. On page 486 of their article they cite, among other guinea pigs, one No. 103, which received 25 milligrams of the residue as a first dose. Ten days later a second dose of 150 milligrams of the residue was given, which did not affect the animal. Five days after the second injection of the residue five cubic centimeters of egg-white dilution were given. Characteristic symptoms developed and the animal died in 40 minutes. After showing that the first dose of the residue was the sensitizing one, and that the second dose was non-toxic, they continue:

"No. 103 suggests another interesting point, *i. e.*, the second dose of the residue did not prevent the animal from splitting up unbroken egg-white given five days later, which would have been the effect had the second dose been a non-fatal one of egg-white."

On studying further the loss of the vaccinating power of the residue, we gave guinea pigs, sensitized by a previous small dose of an active egg-white residue, intraperitoneally a second injection of the residue varying from 200 milligrams to one gram. None of these doses produced anaphylactic symptoms. With the one gram dose there was evidence of abdominal discomfort, but one gram of precipitated egg-white to a 300 gram guinea pig is a formidable dose. Vaughan and Wheeler attribute this absence of toxic properties to the complete separation into the poisonous and non-poisonous portions by their process, while they do not consider at all the effects of the raised temperature by itself on the toxic property of proteins.

An injection of egg-white was given later to test the vaccination. The time between the last dose of the residue and the egg-white varied from 12 hours to 5 days.

In no instance was any evidence of anti-anaphylaxis shown. All the animals acting as the controls were sensitized guinea pigs which had received no second injection of the residue.

Dr. Vaughan told us that he had injected egg-white from one and one-half hours to five days after the second dose of the residue in sensitized guinea pigs, but had never observed any vaccinating effects.

Therefore, it can be stated that no vaccination or protection occurs with the second dose of the residue, although it be large, at least not within the first five days of the injection.

There are two interesting points which these experiments bring up, points which do not quite fit with Vaughan's theory. First, the condition of hypersensitization is produced, according to Vaughan, through the efforts of the body cells to digest the foreign protein, *i. e.*, the unbroken protein or the residue, because both give rise to sensitization, which, according to Vaughan, is increase of proteolytic ferment or zymogen. Yet, a small dose of the unbroken protein suffices to absorb or render inactive the increased ferment, thus producing anti-anaphylaxis. The ferment is used or absorbed in the digestion of the protein. However, experiments

TABLE XII

ABSENCE OF ANTI-ANAPHYLAXIS WITH REPEATED INJECTIONS OF VAUGHAN'S RESIDUE (EGG-WHITE)

G. P. No.	First Injection Intraperitoneally	Interval in Days	Second Injection Intraperitoneally	Symptoms	Time Interval	Third Injection Intraperitoneally	Symptoms	Result
101.....	25 mg. residue	25	5 c. c. egg-white	.....	.....	.....	Typical	Death 10 minutes
102.....	25 " "	25	5 " "	.....	.....	.....	"	Death 20 "
103.....	25 " "	25	5 " "	.....	.....	.....	Severe	Recovery
104.....	25 " "	26	200 mg. residue	None	19 hours	5 c. c. egg-white	Typical	Death 5 minutes
105.....	25 " "	26	200 " "	"	28 "	5 " "	"	Death 2 "
106.....	25 " "	26	200 " "	"	28 "	5 " "	"	Death 2 "
107.....	25 " "	26	200 " "	"	4 days	5 " "	"	Death 30 "
108.....	25 " "	27	1 gm. residue	Abdominal irritation, no anaphylaxis	3 "	5 " "	"	Death 25 "
109.....	25 " "	27	1 " "	Abdominal irritation, no anaphylaxis	3 "	5 " "	"	Death 25 "
110.....	25 " "	27	1 " "	Abdominal irritation, no anaphylaxis	3 "	5 " "	Typical, severe	Recovery
111.....	25 " "	27	1 " "	Abdominal irritation, no anaphylaxis	7 "	.....	Typical, delayed	Death 1 hour, 10 minutes

have proven that even very large doses of the residue are incapable of absorbing or inactivating this ferment, for such is the case with egg-white. Granting with Vaughan that the hypersusceptibility to dead protein, egg-white, and immunity to labile protein, bacteria, are the same, the second point of interest is reached.

Part of Vaughan's specific treatment for typhoid fever is based on this assumption of the vaccinating power or absorption of the ferment by his residue, and yet for egg-white we find it does not exist in even a slight degree.

Vaughan, in his article on the "Specific Treatment of Typhoid Fever,"[3] on page 13 says: "The residue (typhoid) converts the zymogen into an active ferment and at the same time absorbs it. The attraction is between the residue and the ferment. The latter combines with the former more readily when it is free than it does when the residue is still a part of the bacterial cell. When large quantities of the residue are given the activated ferment is all or largely taken up by the free residue and there is no splitting up of the bacterial proteid." And again on page 14, discussing the treatment of a well-developed typhoid fever, when there is an abundant bacteremia, he says: "Larger doses of the residue by absorbing or combining with the specific proteolytic ferment may retard the cleavage of the bacillus, diminish the quantity of poison set free in a certain time and save the life of the patient, but with the effect of prolonging the disease."

However, if the typhoid residue acts as the egg-white residue, as we should be led to expect, no anti-anaphylaxis or vaccination would occur. There would be no union between the typhoid residue and the ferment, therefore from that standpoint the treatment would be of no value. Differences, however, may exist between the two residues, and experiments are now being made on the typhoid. Until they are concluded we can make no definite statement.

The following is a summary of our results:

Comparing the action of Vaughan's egg-white poison to unbroken protein.

1. Inoculations of the unneutralized poison into normal guinea pigs gave the same picture as that of the unbroken protein when inoculated into sensitized guinea pigs, whether the poison was given intraperitoneally or intracerebrally. The filtrate from the neutralized poison injected intracardially also gave typical symptoms.

2. Effect of Heat.—The egg-white was not affected when heated to 100° C. for 15 minutes; heated to 120° C. for 15 minutes its toxic action was reduced, but not completely destroyed.

3. Chloral.—Normal guinea pigs under the influence of chloral were completely protected against one and one-fourth fatal doses of the poison. If two or more fatal doses were given death resulted. Chloral mixed with the poison and then given caused irregular results, which were interpreted as meaning that there is no chemical union of the chloral and poison in vitro. We assume that the chloral protected by union certain vital cells.

4. Lecithin (Egg) Effect on Serum Anaphylaxis.—Emulsified with the serum and the resulting emulsion injected into serum-sensitized guinea pigs; no protection resulted. Lecithin given in doses from 250 to 500 milligrams or more to serum-sensitized guinea pigs protected them from a second injection of five cubic centimeters horse serum given 20 hours later.

5. Lecithin (Egg) Effect on the Poison.—Neither when emulsified with the poison, nor given as a preliminary injection, followed by the poison, 20 hours later, was there any protection afforded. The difference in the protective action of preliminary doses with the serum and with the poison, we think, was due to the absorption by the lecithin of the ferment in the sensitized guinea pig, which causes the splitting of the whole proteins, thus preventing the cleavage and liberation of the poison.

In the case of the poison this cleavage has been done in the retort, and as the lecithin does not protect against the poison itself, death occurs as in control animals. Apparently this supposed absorption of ferment by

the lecithin takes place slowly, for if the lecithin and serum were injected simultaneously death occurred typically.

Eighteen to one hundred hours after the inoculation of 500 milligrams of lecithin the serum-sensitized animals were protected from the second injection of five cubic centimeters horse serum. Thus far these were the time limits, after the injection of lecithin, that we have allowed before giving the second injection.

We assume, with Banzhaf and Famulener, that chloral allows the ferment or zymogen in a sensitized guinea pig to split the protein, but protects the animal's vital cells from the action of the poison, and similarly protects the normal guinea pig from Vaughan's split poison.

The Residue.—1. The sensitizing action of Vaughan's residue (egg-white) differs with different products. Two out of three were inactive. This inactivity may be due to the heat used in the Vaughan method. In fact the loss of the toxic properties of the residue can be fully explained through the action of the heat alone, irrespective of any cleavage of the molecule due to the alcohol and sodium hydroxide used.

2. All vaccinating properties of the active egg-white residue were completely absent. This also would be expected from the prolonged heating to which it has been subjected, which is in complete accord with the effect of heat on whole protein.

The toxic and vaccinating properties are first lost and later the sensitizing properties. The finding of the residues incapable of sensitizing completes the parallel of the effect of heat on unbroken proteins.

If the vaccinating properties of the typhoid residue are lost, as might be inferred from the loss of those of the egg-white, the use of large doses of the typhoid residue, as recommended by Vaughan to absorb the bacteriolytic ferment in typhoid fever showing severe toxic symptoms, would probably not be successful. Experiments now being carried out on the typhoid residue will, we hope, soon permit us to make a definite statement on this point.

Of the theories of anaphylaxis resembling Vaughan's, Richet's theory most closely approaches it. His theory is that anaphylaxis is an intoxication, like other intoxications, with this peculiarity, that the poison which provokes it is due to the union of two substances, toxogenine or the substance contained in the serum of anaphylactized animals, and the substance injected. This union gives rise to a new poison, apotoxin.

Since the phenomena of anaphylaxis are more or less alike in the various intoxications, Richet thinks that the different "apotoxins," which are found at the time of the second injection, are only varieties of one and the same substance, which is the immediate and determining cause of the anaphylactic phenomena.

Vaughan, from his theory, finds that the poisons causing the anaphylactic phenomena in the various intoxications are physiologically identical and chemically probably closely allied.

Vaughan's theory is that this poison is caused by the breaking down of the protein molecule by a proteolytic ferment produced in the body by a preliminary injection.

Vaughan's poison and Richet's apotoxins are, therefore, virtually the same. Vaughan's proteolytic ferment stands for Richet's toxogenine. But while Richet claims to get one and the same substance, apotoxin, by the combination of varying molecules, Vaughan's theory seems more logical; since in chemistry the groups split off in certain cleavage processes are frequently the same, but it is rare to find the combination of varying groups giving the same compound. Therefore, to us, although Vaughan's theory leaves certain facts unexplained, it seems as yet the most closely connected to known and accepted physiological and chemical processes.

[We wish to thank Dr. Vaughan for his generosity and kindness in sending us his split product, and for his courtesy in permitting one of us to finish the work in his own laboratory.]

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THE PRECIPITATION OF DIPHTHERIA ANTITOXIN BY MEANS OF PRECIPITINS

J. P. Atkinson and E. J. Banzhaf, Phar. D.

This work was undertaken partly to check and to review the previous observations by one of us a number of years ago on the precipitation of diphtheria antitoxin from solutions by means of antibodies prepared by injecting diphtheria antitoxic globulin and globulin of normal horse serum into rabbits,[1] partly to determine whether such a method could be used in the further purification of diphtheria antitoxin.

In these earlier experiments it was found that the antibody formed by the injection of globulin of normal serum into rabbits threw out of solution diphtheria antitoxin just as well as the antibody formed by the injection of diphtheria antitoxic globulin.

We undertook these last experiments with the purest diphtheria antitoxin fractionated from the globulin, and serum globulin fractionated in the same way and under the same conditions. The sera were kept at 56° C. for 15 hours to convert, as far as possible, pseudo-globulin into eu-globulin. The diphtheria antitoxin under these conditions remains unchanged with the pseudo-globulin.[2] The solution was then half saturated with ammonium sulphate and the precipitated globulin filtered off. The precipitate was washed with saturated sodium chloride to remove the pseudo-globulin. The sodium chloride solution was precipitated with acetic acid and again filtered and squeezed between filter paper to remove excess of salts, especially ammonium sulphate, and finally dialyzed to free it as completely as possible from salts. Putrefaction was prevented by the presence of chloroform. The dialyzed globulin in the case of the diphtheria antitoxin was highly concentrated and is the antitoxin of commerce to-day.

Both sera, antitoxic and normal, were put through exactly the same process in preparing the globulin for injection.

Injections and Development of Antisera.—Nine rabbits were immunized against diphtheria antitoxic globulin and eight against normal serum globulin as follows:

Antitoxic Globulin, Preparation No. 163.

Injections begun March 7, 1909. Three rabbits injected at intervals of three days.
No. 1—4 injections of 2.5 c. c. produced an immune serum of 1-20,000 strength.
No. 2—6 injections of 2.5 c. c. produced an immune serum of 1-20,000+ strength.
No. 3—9 injections of 2.5 c. c. produced an immune serum of 1-30,000+ strength.
Injections begun May 1, 1909; 2 rabbits subcutaneously.
5 injections, each of 2.5 c. c. every 2 days.
Injections begun December 2, 1909; 2 rabbits.
6 injections, each 2.5 c. c.

Normal Serum Globulin.

Injections begun March 24, 1909; 3 rabbits injected every 3 days.
No. 1—5 injections of 2.5 c. c.
No. 2—5 injections of 2.5 c. c.
No. 3—9 injections of 2.5 c. c.
Injections begun May 1, 1909; 1 rabbit subcutaneously.

[1] J. P. Atkinson, *Med. News*, 1904, lxxxiv, 375.
[2] Banzhaf, *Proc. of the Soc. for Exp. Biol. and Med.*, 1908, vi, 24.

The table shows the results of the addition of immune antitoxic and normal globulin to diphtheria antitoxin.[1]

Preparation of diphtheria antitoxin	Units per c. c.	Dilution with saline sol.	Immune antitoxic globulin	Hours at room temperature	Strength of clear centrifugalized fluid in units[2]	Per cent. of antitoxin in units in precipitate[2]	Precipitate extracted with distilled water in units	Precipitate extracted with glycerine in units[2]	Precipitate emulsified and tested in units[2]
163	700	1 c. c. of 1/10+8 c. c.	+1 c. c.	24 hrs.	700				
163	700	" 1/100+5 c. c.	+1 c. c.	24 "	675	4	none		
163	700	" 1/200+5 c. c.	+1 c. c.	24 "	650	7	"		
163	700	" 1/400+5 c. c.	+1 c. c.	24 "	575	18	"		
163	700	" 1/400	+6 c. c.	24 "	175	75	"		500
			Immune normal glob.						
163	700	" 1/400	+6 c. c.	24 "	175	75	"		500
			Immune antitoxic glob.						
172 B	3000	" 1/3000+	1 c. c.	48 "	300	90	"	725	1500
172 B	3000	" 1/3000+	3 c. c.	48 "	150	95	"	750	1500
			Immune normal glob.						
172 B	3000	" 1/3000+	1 c. c.	48 "	300	90	"	700	2000
172 B	3000	" 1/3000+	3 c. c.	48 "	150	95	"	300	1500
155	850	" 1/200+	No. 3 16 c. c.	48 "	40	95	"	225	500
155	850	" 1/200+	16 c. c.	48 "	40	95	"	225	500
			Gray rabbit						
155	850	" 1/200+	16 c. c.	48 "	170	80	"	200	400
155	850	" 1/200+	16 c. c.	48 "	170	80	,,	200	400

[1]Later experiments showed that the precipitate extracted with sodium chloride yielded some antitoxin. It is evident that a relatively large amount of immune serum must be added to the antitoxin to throw out the antitoxin in anything like quantitative amounts.

[2]Unit strength is calculated on the basis of the undiluted original serum.

5 injections of 2.5 c. c. every 2 days.

Injections begun December 2, 1909; 2 rabbits.

5 injections each of 2.5 c. c.

The rabbits were bled 10 days after the final injection. It was not considered necessary to test the strength of the immune sera after the first tests, since it was all needed for the experimental work.

Two explanations are applicable to these reactions.

1. Diphtheria antitoxin is a globulin which is not changed by heat to a form insoluble in saturated solution of sodium chloride and is consequently precipitated by a globulin antibody of the same nature. We have not yet tried the effect of the addition of an antibody for pure eu-globulin on diphtheria antitoxin.

2. Diphtheria antitoxin is carried down mechanically in the precipitation of the globulin by the antibody as a mordant carries a dye out of solution and holds it.

If the first explanation is true then the combination of the diphtheria antitoxin with the antibody is a comparatively loose one, because normal saline solution and glycerine partially separate them, and the precipitate will neutralize diphtheria toxin.

If the second explanation is true, the precipitation of the globulins by means of a precipitin acts as a very complete and powerful mordant, using the term "mordant" in the broad sense of a clearing agent.

THE PREPARATION OF GONOCOCCAL ANTIGENS FOR THE COMPLEMENT FIXATION TEST

ARCHIBALD MCNEIL, M. D.

The gonococcal antigens first used by Dr. H. J. Schwartz and myself in complement fixation work were prepared from 24 to 48-hour-old cultures of gonococci grown in potato tubes on Thalman's medium to which 2 per cent. of glucose had been added. Two c. c. of physiological saline solution (.85 per cent.) were added to each tube and the cultures scraped off with a platinum loop. The suspension of cocci in saline was heated to 56° C. for half an hour in the water bath, then placed in a mechanical shaking apparatus and agitated for 24 hours at room temperature. The suspension was then centrifugated, the supernatant fluid pipetted off and placed in a sterile bottle, a small drop of lysol being added as a preservative. After standardization the antigen was ready for use. Later we found that simply heating the suspension of gonococci in saline solution at 50° C. for several hours in the water bath gave quite as good results as when the shaker was used.

Antigens prepared in this way, while giving very good results in clinical tests, have a very small range in which they are free from anti-complementary properties; they are unstable, deteriorate rapidly and soon become entirely anti-complementary. They also invariably fix complement with anti-meningococcic sera.

The first attempt to produce a stable gonococcic antigen was made by passing the above preparations through Berkefeld filters. This was accomplished with extreme difficulty, the filters soon becoming completely clogged and the filtrates showed practically no antigenic properties.

Antigens were then made by washing off the growth from 24-hour-old cultures of gonococci with 0.5 per cent. saline solution, and then adding four volumes of distilled water. Various suspensions prepared in this way were subjected to temperatures ranging from —10° C. to +240° C. for different periods of time.

None of these preparations, after passing through Berkefeld filters, showed appreciable antigenic properties; tests made with unfiltered portions prepared at the lower temperatures showed no improvement over antigens made by the original methods, while those prepared at the higher temperatures seemed to have entirely lost their specificity.

Extracts of gonococci made with alcohol, ether and chloroform were then tried. These extracts were evaporated to dryness and the residue taken up with saline solution. All antigens made in this way fixed complement, but, like the previous ones prepared at the higher temperatures, were non-specific.

The first stable and specific antigen was made by washing off 24-hour-old cultures of gonococci grown on plain veal agar (neutral in reaction to phenolphthalein) with distilled water. The suspension of cocci in distilled water was placed in the water bath at 55° C., disintegration of the cocci proceeding rapidly, and was practically complete in about two hours.

On centrifugating only a small precipitate was obtained and the supernatant fluid passed through a Berkefeld filter with comparative facility. The filtrate was clear and nearly colorless and after being made up to the proper tonicity with salt was tested with anti-gonococcic sera and found to actively fix complement. When tested with anti-meningococcic sera no fixation resulted, although the anti-meningococcic sera used fixed complement strongly when tested with meningococcic antigens prepared in the same way.

As salt seemed to be a disturbing element in the preparation of antigens, at the suggestion of Dr. A. W. Williams, of the Department of Health Research Laboratories, cultures were grown on plain veal agar

(neutral in reaction to phenolphthalein), but without the addition of the usual 0.5 per cent. of salt.

On this medium gonococci grew more luxuriantly than on medium containing the additional salt. Cultures of gonococci on this salt-free medium rapidly disintegrated in distilled water, and after centrifugating the supernatant fluid readily passed through Berkefeld filters. Experience has shown that antigens prepared in this way are stable, since they have remained apparently unchanged on standing in the ice box for three months.

One-quarter of a cubic centimeter of these antigens of the strength usually prepared has proven uniformly fatal to rabbits within 12 to 18 hours when injected intravenously. One large rabbit, however, lived for 72 hours after receiving 1¼ c. c. intravenously. This rabbit had been previously immunized to streptococci.

Post-mortem examination of the rabbits showed congestion of all the organs, and a bloody exudate into the peritoneal cavity.

Rabbits injected with successively increasing amounts of the antigens yielded sera that show a high antibody content by the complement fixation test.

Eighty-nine sera from patients having chronic gonorrhœal affections have been tested with antigens prepared as above, with very satisfactory and consistent results, a number of normal human sera being used as controls.

Experiments are now in progress to test the protective power of sera from animals immunized against antigens prepared by this method. This includes not only gonococci, but antigens prepared from many other bacteria. Tests are also being conducted with regard to the comparative toxicity of distilled water extracts of many different bacteria.

It seems essential in preparing bacterial antigens that the culture medium used is neutral in reaction, and that all glassware, filters and other apparatus used are also carefully neutralized, since a very small amount of an acid or alkali seems to destroy their specificity.

Ten of the twelve varieties of gonococci separated by Dr. John C. Torrey, of the Loomis Laboratory, were used in this work.

ON THE USE OF PEPTIC DIGESTION AS AN AID IN TOXICOLOGICAL EXAMINATIONS

James P. Atkinson

GENERAL DISCUSSION

This investigation was undertaken to shorten, if possible, the present methods for the preliminary destruction of tissue in toxicological work. There are several general methods in use for tissue destruction which may be applied after the extraction of the finely divided tissue with acid alcohol to remove alkaloids and synthetic organic compounds. They are all based on the treatment of the tissues with powerful corroding and oxidizing mixtures and require the personal attention of the analyst continually. The method proposed makes use of artificial gastric digestion to break down the protein tissue to a soluble state. The preliminary qualitative work was carried out as follows:

To 5-gm. portions of chopped lean beef .05 gm. of each of the alkaloids and metals given below in the form of soluble salts was added, except in the case of arsenic where arsenious oxide was used; 75 c. c. of artificial gastric juice were added and the digestion carried out at room temperature in Erlenmeyer flasks. The digestion was allowed to progress for two nights and a day, but could have been stopped satisfactorily at the end of 24 hours. The solutions were then made more acid with hydrochloric acid and filtered through wetted papers, and the filtrate shaken in the usual manner with immiscible solvents in the acid, and alkaline, condition. The alkaloids were found in their proper place. The acid condition of the gastric juice converts all alkaloids into their corresponding chlorides soluble in water, and consequently they can be filtered off from the undissolved fats. The fat remaining on the paper may be dissolved with some appropriate solvent, the watery acid solution containing any alkaloid or soluble metallic salt which may have been held by inclusion in the fat. A few little flakes of undissolved protein matter may remain on the paper, together with silver chloride, lead chloride, and any mercurous chloride present, together with synthetic coal tar compounds insoluble in hydrochloric acid. The alkaloids held by the fats and extracted from the undissolved flakes of protein (which will be quite insignificant) may be added to the original filtrate before it is shaken out with the immiscible solvent.

It is important in toxicological analyses that no substances giving alkaloidal reactions shall be formed during the analysis. The result of peptic digestion, not only upon beef, but also upon human tissues, namely, liver, kidneys, spleen, pancreas, heart, stomach, intestine, did not produce any substances giving any characteristic alkaloidal reactions which would mislead the analyst. The following alkaloids have been mixed with chopped beef, digested and recovered: Strychnine, brucine, codeine, morphine, quinine, atropine, coniine, caffein and cocaine. Other alkaloids have not yet been sought by this method. Phenacetin and acetanilid have been successfully recovered qualitatively after peptic digestion. Acetanilid has a marked deterrent effect upon digestion, the exact amount of which has not yet been worked out. The filtered solution, besides the alkaloids, will contain most of the metals. Silver is always found precipitated as the chloride and remains on the filter, as do also lead and mercurous mercury when added in solution as described. The fatty insoluble portion can easily be and should be examined for poisonous coal tar derivatives, besides alkaloids and metallic salts.

The examination for metallic substances was conducted in a somewhat different manner. The digested mass was acidified with nitric acid (about 5 c. c.) after filtration and evaporated to dryness on the water bath. Diluted sulphuric acid was added and the solution again evaporated

to dryness. (If a general examination for poisons were being made the liquid after extraction with immiscible solvents would be acidified with nitric acid and evaporated.) Silver, lead, mercury, copper, bismuth, cadmium, arsenic, antimony, tin, iron, nickel, cobalt, chromium, manganese, zinc, aluminum, barium, strontium, calcium and magnesium were successfully recovered after digestion and identified qualitatively. None of these substances, alkaloids, coal tar compounds, or metallic salts, prevent artificial gastric digestion of meat.

QUANTITATIVE DETERMINATION OF ALKALOIDS

The following nine alkaloids were taken as representative of the group for quantitative separation. The table gives the results of the separations:

TABLE I

Alkaloids	Took	Recovered	Difference	Solvents
Codeine.....	0.01 gm.	0.0099	—0.0001	Ether
Morphine...	0.0100 "	0.0106	+0.0006	Hot Chloroform
Atropine....	0.0100 "	0.0104	+0.0004	Ether
Brucine.....	0.0100 "	0.0104	+0.0004	Ether
Strychnine	0.0100 "	0.0099	—0.0001	Ether
Quinine.....	0.0125 "	0.0130	+0.0005	Ether
Coniine.....	0.0011 "	0.0011	None	Ether
Caffein.....	0.0100 "	0.0087	—0.0013	Chloroform
Cocaine.....	0.0100 "	0.0072	—0.0028	Ether
Cocaine.....	0.0100 "	0.0085	—0.0015	Ether
*Cocaine....	0.0100 "	0.0064	—0.0036	Ether

*After 4 days digestion.

It will be noticed that two of these alkaloids were not completely recovered, namely, cocaine and caffein.

Experiments were carried out to determine the effect of peptic digestion on these alkaloids alone, with the following results:

TABLE II

Alkaloids	Took	Recovered	Difference
Cocaine and Gastric Juice alone.....	0.0100	0.0054	—0.0046
Cocaine and Gastric Juice alone.....	0.0100	0.0077	—0.0023
Caffein and Gastric Juice alone.....	0.0100	0.0095	—0.0005

Not only is cocaine destroyed by digestion, but it also appears to be changed in part in that it gives the characteristic color with chromic acid and hydrochloric acid for novocaine. Time has not yet allowed me to work this out completely.

EXAMINATION FOR METALS

The quantitative examination of protein tissue for metals offers much more difficulty than its examination for alkaloids. Qualitatively all the

metals tested can be found readily after peptic digestion without further manipulation, but for complete quantitative results one of the standard methods must be employed as well after the peptic digestion for complete destruction of the tissue.

The following table gives the results of electrolysis applied to the digested protein containing metals:

TABLE III

(Metals from Chopped Meat and Gastric Digestion)

Metals	Took	Recovered Deposition	Difference	Time
*Lead	0.0385	0.0392	—0.0007	
**Silver	0.0500	0.0495	—0.0005	
Mercury	0.0500	0.0121	—0.0379	45 hours
Mercury	0.0500	0.0217	—0.0283	45 hours
Copper	0.0500	0.0485	—0.0015	35 hours
Cadmium	0.0500	0.0324	—0.0176	45 hours
Tin	0.0330	0.0051	—0.0279	
Tin	0.0330	0.0063	—0.0267	30 hours
Zinc	0.0500	0.0561	+0.0061	30 hours
Cobalt	0.0500	0.0503	+0.0003	30 hours
Nickel	0.0492	0.0497	+0.0005	30 hours
Iron	0.0500	0.0497	—0.0003	†15 hours
Arsenic	0.0500	No reaction in Marsh apparatus after		50 hours
Antimony	0.0500			

*Lead was determined as the sulphate after ashing the tissue residue.

**Silver was determined as the chloride after ashing the tissue residue.

†Electrolysis was run over night.

Mercury, cadmium and copper have been added to Witte's peptone and recovered practically quantitatively as is shown in Table 4:

TABLE IV

(Metals from Wittes' Peptone Solution)

Metal	Took	Recovered Deposition	Difference	Time
Mercury	0.0500	0.0470 H_2SO_4Sol.	—0.0030	5 hours
Mercury and Copper	0.0750			
	0.0500=0.1250	0.1215 H_2SO_4Sol.	—0.0035	*16 hours
Copper	0.0500	0.0568	+0.0068	*16 hours
Copper	0.0500	0.0470	—0.0030	not noted
Mercury and Copper	0.0250			
	0.0500=0.0750	0.0790	+0.0040	20 hours
Cadmium	0.0500	0.0583	+0.0083	5 hours

*16 hours indicates that the electrolysis was run over night.

The cause of the prevention of the deposition of the metals by electrolysis, judged by these results, seems to be more complex than the simple presence of peptones could explain, since Witte's peptones do not interfere, but, on the other hand, in the case of mercury, cadmium and copper, assist in the deposition.

Work is being carried out to determine the cause of this interference in the deposition in the digested protein-metal substance.

Animal experiments are being made to test the therapeutic value of these protein-metal compounds.

SUMMARY

1. The peptic digestion of protein substances facilitates the removal of alkaloids and greatly assists in the destruction of protein in metal examinations.

2. Further destructive treatment of the digested protein is necessary in quantitative determinations of metals.

3. The time required for analysis in toxicological cases is materially shortened.

MUNICIPAL DISINFECTION IN NEW YORK CITY AS RECENTLY REORGANIZED.

M. C. Schroeder, M. D.
Assisted by B. Junger, Ph. G.

Brief Résumé of Former Methods of Disinfection.
Study on New Methods of Room Disinfection.
Book Disinfection.
System Adopted for Controlling Practical Disinfection.
Résumé and Conclusions.

Brief Résumé of Former Methods of Disinfection in New York City.—Sulphur was the standard material used for the disinfection of rooms up to 1895, four pounds of roll sulphur being burned to disinfect 1,000 cu. ft. of space. This was gradually discarded in favor of formaldehyde gas in 1902. At first this was generated from formalin by heat from a flame; later a combination of a solution of formaldehyde, aluminum sulphate solution and lime was utilized, one pound of lime (fresh unslacked granular) being allowed for every 1,000 feet of space. Over this was poured a mixture of six ounces of formaldehyde, 40 per cent. strength, and two ounces of a saturated solution of aluminum sulphate; a fairly rapid evolution of the gas resulted.

This method was open to several objections, one of the most important being the excessive weight of the materials; another was the irregularity of results. The conditions in New York City required that the disinfectors should carry by hand the chemicals and necessary utensils required for disinfection, and the weight of this material amounted to 20 lbs. for every 10 disinfections of 1,000 cu. ft. of space. It was therefore necessary to adopt or devise some combination of chemicals which would prove satisfactory for the purposes of municipal disinfection. In all, some 55 formulæ were tested; first, as to the efficiency; second, as to the time required for a compound to disinfect properly; third, as to the cost; fourth, as to the danger of fire; fifth, as to ease of transportation, the last factor being important owing to the frequent long distance from the base of supplies.

(*a*) *Technic Employed.*—The limit of time selected was four hours, this period having been found to impose the least hardship upon the many people whose small apartments require disinfection, and who frequently have to spend that time in the parks and streets. The method of testing the effectiveness of disinfection was by means of threads, dipped in 24-hour cultures of B. coli and B. pyocyaneus dried in air and placed on cards, according to the Wilson method, or in tissue paper envelopes. After the test these threads were immediately removed with sterile forceps and placed in test tubes containing a suitable medium—B. coli in lactose neutral red fermentation tubes, and B. pyocyaneus in the synthetic medium later described. These tubes were then incubated at 37° C. for three days, after which readings were made.

The formulæ can be grouped as follows:

1. Mixtures of lime, potassium permanganate and formaldehyde and different salts in varying proportions.
2. Potassium permanganate and formaldehyde in different proportions.
3. Potassium permanganate, sand and formaldehyde in different proportions.
4. Potassium permanganate, paraformaldehyde and water in different proportions.
5. Solidified formaldehyde and heat.

The standards of efficiency, time limit, cost and weight eliminated the greater part of these formulæ.

(*b*) *Preliminary Tests.*—Many of the combinations were given preliminary trials to note any possible defects in chemical reaction, bulk and cost, and some of those rejected for any of those reasons are grouped together in Table 1.

TABLE 1.

FORMULÆ REJECTED AFTER PRELIMINARY TRIAL.

Lime	Potassium Permanganate	Formaldehyde	Water
Ounces per 1,000 Cubic Feet	Ounces per 1,000 Cubic Feet	Ounces per 1,000 Cubic Feet	Ounces per 1,000 Cubic Feet
5.0	2.5	5.1	5.1
4.8	2.5	5.1	5.1
...	9.0	10.0	5.0
...	8.0	10.0	5.0
...	10.0	7.0	3.5
...	20.0	6.0	3.0

A series of experiments were also tried in which sand was used as a mechanical means of checking the violent reaction of the potassium permanganate but the formulæ were rejected as too much formaldehyde was retained in the residue.

(*c*) *Final Tests.*—Those compounds which passed the preliminary trial were then tested in room disinfection and subdivided again as follows:

1. Compounds which required five to six hours to complete the destruction of the testing organism, but fulfilled the requirements as to weight, cost, etc. (Table 2.)

TABLE 2.

Formula giving good result in 6 hours' time:

Material.	Ounces per 1,000 cu. ft.	
$K_2M_2O_8$	5	Tested May 29, 1910.
CHOH	6.8	Amount of gas set free by this method, 41 per cent.
H_2O	2.5	Volume of gas tested by Lockerman & Kroner's apparatus.
Lime	2.5	Size of room 1,129 cu. ft. of space, time 6 hours.

24-hour cultures of B. pyocyaneus, B. coli used on threads in envelopes:

Cultures.	Growth.	No Growth.	
B. Pyo.	..	10	Total number of tests exposed, 20.
B. Coli	..	10	Number of tests showing growth, 0.
B. Pyo.	5		Number of tests not showing growth,
B. Coli.	5		20-100 per cent.

As this formula seemed to meet all the requirements except the time limit, it was repeatedly tested in rooms of different size to ascertain if this factor could not be reduced, but without any change in the results.

2. Those compounds which resulted in the destruction of the testing organism after four hours' exposure, but were rejected on account of excessive weight, cost, etc. (Table 3.)

TABLE 3.

FORMULÆ* GIVING RESULTS IN 4-HOUR ROOM, DISINFECTION, REJECTED ACCOUNT OF EXCESSIVE WEIGHT, COST, ETC.

Number of Ounces	Lime	Potassium Permanganate	Formaldehyde	Water	Oxalic Acid 1–10
Test	Ounces per 1,000 Cubic Ft.	Ounces per 1,000 Cubic Ft.	Ounces per 1,000 Cubic Ft.	Ounces per 1,000 Cubic Ft.	Ounces per 1,000 Cubic Ft.
....	.....	20.0	20.0	10.0	.....
....	.....	10.0	9.0	45.0	.....
....	10.0	5.0	10.0	5.0	5.0
....	8.0	7.0	5.0	10.0	5.0
....	3.0	3.05	5.6	3.0	.....
....	6.0	.....	5.0	5.0	5.0

*Other mixtures having a reduction of 20 per cent. in the amount of potassium permanganate or of 10 per cent. of the formaldehyde and water gave equally good results but had the same objections.

3. Those compounds which brought about a successful disinfection in four hours' time and were otherwise satisfactory. (Table 4.)

TABLE 4.

Compounds causing death of the testing organism in four hours and meeting all the requirements as to cost, ease of transportation, etc.

Formula 1.—Potassium permanganate, 30 grams, or 1 ounce; paraformaldehyde, 75 grams, or 2.5 ounces; water 90 grams, or 3 ounces.

Formula 2.—Fumigator compound, solidified formaldehyde, carbolic acid, glycerine, etc.

In this last division there were only two compounds to be tested. First, a mixture of paraformaldehyde, potassium permanganate and water, and, second, a solidified formaldehyde compound, consisting of formaldehyde, carbolic acid, glycerine and other chemicals. The compound being volatilized by the aid of a flame.

At this time also an extended series of analyses were made for the purpose of ascertaining the amount of formaldehyde gas evolved by these various compounds, as well as the amounts remaining in the residue. Thanks are due to Dr. Banzhaf and Miss B. Junger for their aid in this direction. The evolution of gas was tested by the Lockerman and Kroner method, and also by means of the Kjeldahl flask. The residues were also tested for the amount of residual formaldehyde. A large number of tests were made, and the conclusions drawn were as follows:

1st. That in the lime, aluminum sulphate and formaldehyde method of disinfection the evolution of gas is less complete than has generally been supposed. This also holds good for the potassium permanganate and liquid formaldehyde method.

2nd. That with the paraformaldehyde and permanganate method the results obtained were more satisfactory, the amount of gas evolved running as high as 87 per cent.

The Method of Testing Residue for Residual Formaldehyde.—After the evolution of gas had ceased, the residue was carefully weighed and thoroughly mixed. Fifty grams were then taken and washed thoroughly with water, filtered and the filtrate diluted to 500 c. c. with distilled water. Of this dilution 20 c. c. were taken for analysis and tested according to the

iodine-thio-sulphate method in order to ascertain the amount of formaldehyde present. The remainder of the residue was then dried by means of a water bath and desiccator, the loss in weight noted, and from the resulting figure the amount of formaldehyde present in the entire residue calculated. Records of the results of some of the tests are given in the pages at the end of the section.

Much work has recently been done both here and abroad with combinations of paraform and potassium permanganate and water. Lockerman and Kroner have done considerable work in this direction. In testing the paraformaldehyde-permanganate method for municipal disinfection, we found that the following amounts of the chemicals made a good mixture and gave sufficient formaldehyde to disinfect test threads in 1,000 cubic feet of space in four hours' time:

	Grams.
Paraformaldehyde	30
Potassium permanganate	75
Water	90

The two chemicals are put up separately in small boxes containing sufficient material to disinfect 1,000 cubic feet of space, and carried by the disinfector to the infected house. Here the two chemicals are thoroughly mixed in small deep tin pans (½-quart size), then the water is added and the mixture again thoroughly stirred. The evolution of gas is comparatively slow in starting, and this gives the disinfector time to leave the room and seal the door properly without being annoyed by the fumes of formaldehyde. The evolution of gas is practically completed in 5 to 10 minutes.

The second method, which was found most excellent in many respects, was carried out by means of a fumigator. This fumigator consists of a receptacle or deep cup containing a layer of paraffin and a circular wick to which air is admitted through spaces cut in the sides. In the upper part of this receptacle another cup containing 48 grams of the mixture of solidified formaldehyde is placed. The candle, when ignited, is set in a shallow pan containing cold water. The evolution of gas takes place with quiet bubbling, the entire process being completed in one-half hour.

Both of these methods show distinct advantages over that formerly in use. First, on account of the greater amount of gas evolved; second, because of the light weight of the chemicals used, thus giving a better disinfection and avoiding one of the great objections to the former method, where a disinfector had to carry from 50 to 100 pounds, often returning for as much more in order to complete a day's work.

The following table of sufficient materials for 10 disinfections of 1,000 cubic feet space illustrates the difference in weight of outfits to be carried:

1. Lime and Formaldehyde Method.

	Lbs.
Lime	10
Formaldehyde and aluminum sulphate solution	6.25
Pans	.5
Cartons	1.35
Bottles for carrying solution	2.5
	20.60

2. Paraform and Permanganate Method.

Paraform	10	ozs.
Potassium permanganate	25	"
Cartons	3.33	"
Pans	13	"
	3.20+	lbs.

3. Fumigator Method.

2.6 lbs.

In any method of disinfection involving the use of heat, one of the most important considerations is, of course, the avoidance of the risk of fire. In our experiments many methods were rejected on this ground alone, and this was one of the most important factors determining the final choice of a routine method. A slight risk of this kind is attached to combinations of paraformaldehyde and potassium permanganate, but the advantages of such a combination are so obvious that it is worth while to find some means by which it can be used with safety. The Germans found that intense heat was evolved by pouring small quantities of water upon large masses of paraform and permanganate, thus bringing about very rapid action, but at the same time producing a vivid flame. They guard against this danger by first mixing together the permanganate and water, to which soda is added in small quantities, and last of all the paraformaldehyde. They further recommend the use of small vessels for containers, thus dividing the charge and effectually guarding against any danger from fire which might otherwise be present.

As the result of the work of others and the investigations reported here, the Board of Health decided in October, 1910, to adopt the paraform-permanganate method for their routine room disinfection, and this method has been utilized with satisfactory results during 1911. The tests are given in detail at the end of this article.

(*d*) *Book Disinfection.*—A question raised in the course of these experiments was as to the efficiency of the methods usually applied to the disinfection of books, the opinion having been prominently advocated by some not familiar with previous investigations that the complete destruction of books is necessary after contact with a contagious disease. It was thought best, therefore, to make a series of experiments to retest this matter. The method in common use consists of the exposure of books or papers to formaldehyde gas, formaldehyde gas and heat, or heat alone.

The following work was planned and carried out as follows:

1. Exposure of books inoculated with bacteria to formaldehyde, aluminum sulphate and lime method and heat in varying amount for three hours.

2. Exposure of books inoculated with bacteria to formaldehyde, aluminum sulphate and lime method at room temperature for 24 hours.

3. Exposure of books inoculated with bacteria to the action of dry heat alone at a temperature of 50°-55° C. for three hours.

4. Exposure of books inoculated with bacteria to the action of dry heat alone at a temperature of 50°-55° C. for 24 hours.

In this work I was assisted by Miss B. Junger and Miss Schweitzer, assistants at the Laboratory. The books used were ordinary library and school books, and the tests were conducted in the following manner: One-half of the books were closed and the other half had the covers fastened back so that the gas could penetrate freely. At intervals of 10, 20 or 100 pages, a page was divided into four parts and these parts subdivided into squares, the parts being labeled as follows: upper outer corner, upper inner corner, lower outer corner, lower inner corner, these portions of the pages being most liable to contamination. In the squares thus marked the surface of the page was directly smeared with a 24-hour culture of B. pyocyaneus and a 24-hour culture of B. coli. In a second square a thread inoculated with similar cultures was fastened with a drop of paraffin. After the tests, these threads were immediately removed with sterile forceps and inoculated into test tubes, the B. pyocyaneus in the synthetic medium and B. coli in lactose neutral red fermentation tubes.

The paper squares were then cut out and dropped into similar tubes, and controls were made. All the test tubes were inoculated at 37° C. for three days, after which readings were made.

Conclusions.

1. Ordinary routine room disinfection disinfects only the surfaces of exposed pages and covers of books.

2. Reviewing the formaldehyde, aluminium sulphate and lime method, it appears that a large quantity of formaldehyde—44 ozs. per 1,000 cu. ft.—

together with heat, 50°-55° C., will destroy ordinary bacteria in books in three hours' time if the books are opened and the leaves spread apart.

3. The various tests by heat alone show that a short exposure, three hours, 49°-51° C., does not destroy all pathogenic bacteria.

4. A 23 hours' exposure at 50°-55° C. will destroy bacteria, but is apt to warp the books by drying them excessively.

5. A three-hour exposure at 55° C. to moist heat and formaldehyde will kill the bacteria, and beyond a slight spreading of the leaves do no damage to the books.

Controlling Disinfection.

The control of disinfection by means of tests is of comparatively recent origin, Koch, in 1887, being the first to mention its use. He selected the B. prodigiosus and the B. pyocyaneus, and grew them on a potato medium, testing disinfection by exposing both culture medium and organism to the action of the disinfectant.

The method of testing disinfection formerly in use in New York City was that known as the Wilson method, having been elaborated by Dr. R. J. Wilson, then Assistant Director in the Research Laboratory.

The method depended for its efficiency on the color produced by B. pyocyaneous when grown in bouillon. The manner of using it was as follows: Fine cords were inoculated with this organism and fastened by means of a drop of paraffin to a cardboard folder. These folders were sent out two or three times a week to the various boroughs and distributed to the disinfectors, who placed the opened folders in the rooms to be disinfected and left them there during the period of disinfection. Upon the completion of disinfection they were removed by the drivers who called to remove the bedding for steam sterilization, and were brought to the laboratory.

Here the threads were removed and placed in both tubes and incubated for five days. They were then examined, and those showing the color reaction were marked positive and a record made. This most excellent method was in use a number of years. It was found, however, that these tests were easily contaminated in the process of handling, and might thus lead to unfair deduction, first, as to the character of the materials used; second, as to the mode of disinfection; third, as to the thoroughness of the process.

Studies were begun in 1908, with a view of obviating these drawbacks. Comparisons were made as to the advantages of envelopes, as compared with the cardboard folders, for holding the test threads. About 30 different kinds of envelopes were tried, varying in size, form, position of opening, and quality of paper, the latter ranging from tissue to manila, glazed, unglazed, etc.

An oblong envelope, 4¼ x 3 inches, made of the quality known as French White tissue paper, was finally decided upon as affording the best protection from accidental contamination, while at the same time not interfering with the action of the disinfecting gas upon the threads.

The next step after the choice of the envelopes was to find some way of obviating the frequent loss or destruction of the tests by foreigners unacquainted with their importance. For this purpose the words, "Poison, do not destroy," were printed on the outside of the envelopes in large red letters in four different languages (see Fig. 1). All other necessary data was printed in black ink. This method of printing has proved so efficient that very few tests are now lost or carelessly destroyed.

Modification of Medium.

Owing to the inhibiting effects produced upon the organisms when these were not entirely killed by the disinfecting gas, four or five days were required for the color to develop in the broth medium used. Atten-

tion having been directed to the results obtained by Sullivan, Frankel and Vosges, and later by Ayer, in the color production by bacteria on synthetic media, studies in this direction were begun in 1908.

Numerous experiments were made with many forms of synthetic media containing various combinations of the substances recommended by those men, such as asparagin, ammonium lactate, magnesium, sodium phosphate, sodium chloride, etc.

The formula given by Ayer and ascribed to Frankel and Vosges consists of:

	Grams.
Asparagin	4
Sodium phosphate	2
Ammonium lactate	6
Sodium chloride	5

Mix thoroughly and add 1,000 c. c. of water.

After trying many modifications of this medium, it was found that it could be improved by the substitution of sodium lactate for ammonium lactate, as the former is more stable. It was also found that better results

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DEPARTMENT OF HEALTH

DISINFECTION TEST

To be called for by Driver

POISON! DO NOT DESTROY

Gift! Zerstöre es nicht

VELENO! Non Lo Distruggete

גיפט! צושטערט עם ניט

Test Issued .....

Date and Time Exposed .....

Name .....

Address .....

Disease .....

Disinfector .....

Laboratory Test .....

Result .....

FIG. 1.—Face of envelope.

were obtained after careful neutralization, a point not mentioned by Ayer. The following formula was finally adopted:

	Grams.
Asparagin	4
Sodium lactate	6
Sodium phosphate (ortho)	2
Sodium chloride	5
Water	1,000 c. c.

The method of preparing this medium is as follows: The salts are finely powdered, and dissolved separately in 50 c. c. to 100 c. c. of water. When thoroughly dissolved the solutions are added to the remainder of the water and the mixture, if necessary, is rendered alkaline to litmus by the addition of NaOH. It is then filtered and tubed into 3 x ⅜-inch tubes. After sterilization on two consecutive days in the Arnold Sterilizer, the medium is tested for pigment production by inoculating one of the tubes with the Bacillus pyocyaneus.

If properly made the medium is a clear, colorless fluid. When inoculated with B. pyocyaneus the color change presents itself in 24 hours' time as a clear light green band in the upper portion of the test tube, which stands out sharply from the colorless fluid below. This band gradually increases in depth until the entire content of the tube is a bright green. At the end of a week the color loses its clear green tint and becomes yellowish green and muddy. The medium thus gives a vivid color reaction. It was also found to have the added advantages of inhibiting the growth of non-chromogenic organisms.

System Adopted for Testing and Controlling Practical Disinfection.

While these studies were progressing, the following system of testing municipal disinfection was formulated and adopted. The main points of this system are as follows:

1. An oblong tissue paper envelope 3½ x 4 inches, containing the test thread and stamped with all necessary indications for data (see Fig. 1).

2. A driver's report card, 3 x 5 inches (Fig. 2), which is inclosed in stout manila envelope and on which is recorded the receipt of the test.

DRIVER'S DAILY REPORT—DISINFECTION TESTS.

Driver ____ Date ____

Name	Address	Test col'ct'd	No test left	Test lost

FIG. 2.

3. A disinfector's card, 4 x 6 inches, which, when ready for final filing, gives a simple, complete history of the test from the date of its issue through its final examination at the laboratory (see Fig. 3).

DEPARTMENT OF HEALTH.

REPORT ON DISINFECTION TESTS.

Disinfector..... *Week beginning*.....

ADDRESS	Disease	Test Issued	Test Exposed	Received at Desk	Tested	Disinfect. Success.	Disinfect. Unsuccess.	No. Tests Collected	Old Tests Used	

FIG. 3.

4. A bacteriologist's report (see Fig. 4) which gives in a concise manner a résumé of the work done by each disinfector during the entire week. These records are kept until the end of the week and are then sent to the respective boroughs, where they are filed. The test envelope is filed with the case records. The disinfector's card is filed with his rec-

DEPARTMENT OF HEALTH

DIVISION OF LABORATORIES

Bacteriologist's Report on Disinfection Tests. *Week Ending*.....

DISINFECTORS	Diphtheria	Scarlet Fever	Measles	Tuberculosis	Small Pox	Typhoid	Cerebro-Spinal Meningitis	Miscellaneous	Disinfectant Successful	Disinfectant Unsuccessful	Old Tests Used	No. Tests Collected

FIG. 4. (Face)

BOROUGH REPORT

BOROUGH	Number of Tests Sent	Number of Used Tests Returned	Unused Tests Returned				
MANHATTAN							
BROOKLYN							
THE BRONX							
QUEENS							
RICHMOND							

FIG. 4. (Back)

ord and the bacteriologist's report in the Assistant Sanitary Superintendent's office. The whole outfit, assembled, is shown in Fig. 5.

The technic in detail is as follows: No. 36 cotton is cut into inch lengths, placed in a Petri dish, and covered with a 48-hour broth culture of B. pyocyaneus. They are left for two or three minutes, or until they are thoroughly saturated, then removed to filter paper in another covered

Petri dish and left to dry for five minutes. When dry they are placed in the tissue envelopes (see Fig. 1).

The envelope is dated and sealed and sent each day to the disinfector, who places it in the room which is to be disinfected, attaching it to the wall by means of its gummed flap.

After disinfection, the driver who calls for the bedding takes up the test, placing it in the manila envelope and entering it upon his card (see Fig. 2). This envelope is then returned to the laboratory, where the tests and receipt card are compared and any discrepancy noted. They are also compared with the daily list of disinfections done, and checked back in the following manner:

V —Test received.

X —Test not received.

(X)—Test received late, noting delay in transmission.

The test envelopes are then stamped with date of receipt, and the threads are removed and placed in the synthetic medium. The tubes are

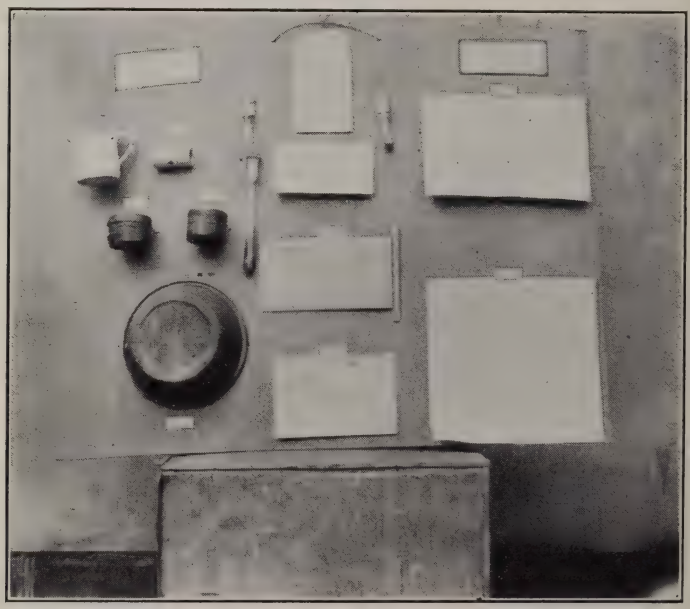


Fig. 5.

incubated for 48 hours and the color reaction noted and the results entered upon the test envelopes. Upon completion of this process the envelopes are sorted. They are then entered upon the disinfector's cards. Account is also kept of the number of tests sent out and unused tests returned. At the end of the week a bacteriologist's report is compiled, which shows at a glance the work of each disinfector, the number of cases of each disease for which disinfection was performed, the number of successful and unsuccessful disinfections, the number of tests lost, etc. (see Figs. 5 and 6).

Résumé and Conclusions.

In conclusion, a recapitulation of the method of disinfection and its control used in the New York Department of Health at present is as follows:

Disinfection of Rooms.—This is done by means of the paraformaldehyde and potassium permanganate method. With this method we allow the following amount for 1,000 cu. ft. of space: Paraformaldehyde, 30 grams, or 1 ounce, potassium permanganate, 75 grams, or 2½ ounces; water, 3 ounces.

The chemicals are put up separately in pasteboard boxes—the paraformaldehyde is placed in a round straw-colored box 1⅜ inches high and 2 inches in diameter, the potassium permanganate is packed into a round box 1⅜ inches high and 2¼ inches in diameter. These boxes are then covered with melted paraffin. A small aluminum cup holding exactly 3 ounces of water is also provided, together with a small pan made of black iron. This pan is 4¼ inches wide at the bottom, 2 inches deep, and 6¼ inches wide at the top (see Fig. 5).

When the premises to be disinfected are reached, the room is sealed, the disinfection test envelope is hung up at least six feet above and six feet distant from the disinfecting mixture. The contents of one straw-colored box and a black box are mixed together in the pan, and the three ounces of water are added. The mixture is again thoroughly stirred, the door of the room is sealed and the room is left for four hours, when disinfection is said to be completed. The room is then opened and the test is removed and sent to the laboratory. If there is bedding to be removed this is sent to the disinfecting station to be disinfected with live steam.

The Disinfection of Books.—The books are opened up so that the leaves are separated. They are then placed in a small steam chamber and 6 ounces of paraform, together with 24 ounces of water and 18 ounces of permanganate of potassium for 1,000 cu. ft. is allowed. The heat is regulated so that it remains at a pretty even temperature of about 55° C., and the books are then left until disinfected.

The Control Test of disinfection is done by means of thread infected with a broth culture, B. pyocyaneus, which is placed in the tissue paper envelopes and then placed in the room or among the articles to be disinfected. Upon conclusion of the disinfection, the tests are collected by the drivers collecting the bedding, and delivered to the laboratory, where the bacteriological test is carried out as previously outlined. Then the reports are forwarded to the various divisions.

All these methods have been in use for the past year and have proved satisfactory.

In closing I wish to thank most heartily Dr. William H. Park, General Director, Division of Laboratories, for the many helpful suggestions and unvarying and kindly interest he has shown in these studies.

Details of a Few of the Tests Carried Out in the Investigations.

Test 1.—Paraform, 30 gms.; Potassium permanganate, 70 gms.; water, 86 gms.

Size of room, 1,129 cu. ft.

Length of exposure, 4 hours.

Bright sunshiny day.

The floor, made of concrete, had just been washed. Twenty-four hour bouillon cultures of B. coli and B. pyocyaneus were used on cotton threads. Threads were inoculated by immersion in these cultures and then dried on filter paper. They were then placed in envelopes made of tissue paper, and suspended in the center of room at least five feet distant from the pan. Ten threads of each were used, and five controls of each organism were made and kept until the room was opened and the envelopes containing the test threads were removed. The test threads, as well as the controls, were at once placed in tubes of suitable media and the tubes incubated at a temperature of 37° for three days, after which readings were made.

RESULTS OF TEST NO. 1.

Culture.	Exposed Threads.	Growth.	Growth.
B. Coli	10	..	10
B. Pyo.	10	..	10
	Control on Above.		
B. Pyo.	5	5	..
B. Coli	5	5	..

Total number of tests exposed, 20.
Total number of tests showing growth, none.
Total number of tests showing no growth, 20—100 per cent.

Under as nearly as possible identical conditions, this experiment was repeated on August 13th, 15th, and 18th. The humidity was not tested on the 13th, but was respectively 90 and 91 per cent. on the last two days. The results were identical with those in the above table. All bacteria on exposed threads were destroyed.

Test 5.—More extended test, where bacteria were exposed in different portions of the room:

Paraform, 29.23 gms.; permanganate, 70 gms.; water, 86 gms.
Size of room, 1129 cu. ft.; 2 doors, 1 window.
Length of exposure, 4 hours.
Clear day; relative humidity, 88 per cent.
Entire weight of chemicals, 186 gms.
Weight of residue after test, 104.68 gms.
Amount lost in test, 81.32 gms.
Loss in weight of residue, 32.32 gms.
Amount of formaldehyde in residue, .0304 per gm.
Entire amount of formaldehyde in residue, 11.25 per cent.
Amount of formaldehyde given off, 88.75 per cent.

In this test the threads were exposed on both cards, according to the Wilson method, and in envelopes according to the method given above. They were also placed in various parts of the room to ascertain distribution of the gas.

How Exposed.	Where Exposed.	B. Pyo. Grwth.	B. Pyo. No Grwth.	B. Coli. Grwth.	B. Coli. No Grwth.
On cards, Wilson method.	On floor 5 ft. distant....	..	10	1	9
In envelopes..	On floor, 5 ft. distant....	..	10	..	10
On cards.....	Upon top of closet, 6 ft. above and 6 ft. distant	..	10	..	10
On cards.....	Upon wall 8 ft. above, 6 ft. distant from pan in left-hand corner of room	..	10	1	9
In envelopes..	Upon wall 8 ft. above, 6 ft. distant from pan in left-hand corner of room	..	10	..	10
On cards.....	On floor under table.....	..	10	1	9
In envelopes..	between closet and wall.	..	10	3	7
On cards.....	On line center of room..	..	10	*1	9
In envelopes..	On line center of room..	..	10	..	10
On cards.....	On floor 6 ft. distant from pan	..	10	..	10
In envelopes..	On floor 6 ft. distant from pan	..	10	..	10

* Growth showed on sixth day after test tubes had been accidentally tipped.

Summary of Total Number of Tests.

How Exposed.	Number.	Growth.	No Growth.
On cards	120	4 = 3.3 per cent.	115 = 94.88 per cent.
In envelopes	120	3 = 2.4 per cent.	117 = 97.5 per cent.

Test 6.—In this test the penetrative power of the gas was tested and the amount of the chemicals was increased 1-10.

Paraform, 33 gms.; potassium permanganate, 75 gms.; water, 90 gms.
Size of room, 1,129 cu. ft; 1 window, 2 doors.
Length of exposure, 4 hours.
Relative humidity, 98%.
Entire weight of chemicals, 198 gms.
Loss in weight of entire residue, 77.02 gms.
Actual weight of formaldehyde, 29.7 gms.
Weight of residue after test, 120.98 gms.
Amount of formaldehyde in residue, 3.48:11.71 per cent.
Amount of formaldehyde released, 88.29 per cent.

Exposed on line in center of room, 6 ft. above, 6 ft. distant from pan.

	B. Coli.		B. Pyo.	
How Exposed.	Growth.	No Growth.	Growth.	No Growth.
On cards	..	5	..	5
Envelopes	..	5	..	5
On cards, in fold of blanket pocket..	..	5	..	5
In envelopes	..	5	..	5
On cards, 2 fold blanket pocket	..	5	..	5
In envelopes, 2 fold blanket pocket...	..	5	1	4

Exposed near window, 8 ft. above, 5 ft. distant.

	B. Coli.		B. Pyo.	
How Exposed.	Growth.	No Growth.	Growth.	No Growth.
In envelopes	..	5	..	5
On cards	..	5	..	5
In envelopes, in 1 fold blanket pocket	..	5	..	5
On cards, in 1 fold blanket pocket....	1	4	..	5
In envelopes, in 2 fold blanket pocket	..	5	..	5
On cards, in 2 fold blanket pocket...	..	5	..	5

Exposed in angle formed by closet and wall, 5 ft. distant.

	B. Coli.		B. Pyo.	
How Exposed.	Growth.	No Growth.	Growth.	No Growth.
On cards	..	5	..	5
In envelopes in 1-fold blanket pocket.	..	5	..	5
On cards, in 1 fold blanket pocket....	..	5	..	5
In envelopes, in 2 fold blanket pocket	1	4	..	5
On cards, in 2 fold blanket pocket...	3	2	..	..

On floor, in angle formed by closet and wall, 7 ft. distant.

	B. Coli.		B. Pyo.	
How Exposed.	Growth.	No Growth.	Growth.	No Growth.
In envelopes	..	5	..	5
On cards	..	5	..	5
In envelopes, in 1 fold blanket pocket	..	5	..	5
On cards, in 1 fold blanket pocket...	..	5	..	5
In envelopes, in 2 fold blanket pocket	..	5	..	5
On cards, in 2 fold blanket pocket...	..	5	..	5

Exposed on floor near window.

How Exposed.	B. Coli. Growth.	B. Coli. No Growth.	B. Pyo. Growth.	B. Pyo. No Growth.
In envelopes	..	5	..	5
Cards	..	5	..	5
Envelopes	..	5	..	5
On cards, 1-fold blanket pocket	..	5	..	5
In envelopes, 1-fold pocket	..	5	..	5
On cards, 2-fold blanket pocket	..	5	..	5
In envelopes, 2-fold blanket pocket	..	5	..	5

Summary of tests:

How Exposed.	Number.	Growth.	No Growth	Per cent. Growth.	Per cent. No Growth.
On uncovered cards	50	1	49	2	98
On cards under 1-fold blanket	50	1	49	2	98
On cards under 2-fold blanket	50	4	46	8	92
In envelopes	50	..	50	..	100
In envelopes under 1-fold blanket	50	..	50	..	100
In envelopes under 2-fold blanket	50	2	48	4	96

Book Disinfection.

Test 1.—Formaldehyde, aluminum sulphate, lime method and heat; 3 hours' exposure.

Amount of formaldehyde estimated 40 per cent. per 1,000 cu. ft., 35 oz.

Size of room, 176 cu. ft.

Temperature ranged from 49° to 50° C. for 1 hour, and from 50° to 51° C. for two hours.

One-half of the tests were incubated at once; the remaining half were kept 24 hours to determine death or attenuation of the testing organisms.

Book used: Vol. 1—560-page, board and linen covers, book open, covers held back.
Vol. 2—195-page, board and linen covers, book open, covers held back.
Vol. 3—575-page, board and linen covers, book open, covers held back.

Incubated at once:

Vol. 1, 560 pp., open:

Bact.	Growth. Thread	Growth. Paper.	No Growth. Thread.	No Growth. Paper.	Doubtful.	Total.
B. Pyo.	2	4	15	13	..	34
B. Coli	6	..	12	18	..	36

Vol. 2, 195 pp., open:

Bact.	Growth. Thread	Growth. Paper.	No Growth. Thread.	No Growth. Paper.	Doubtful.	Total.
B. Pyo.	3	2	7	8	..	20
B. Coli	6	1	4	9	..	20

Vol. 3, 575 pp., closed:

Bact.	Growth. Thread	Growth. Paper.	No Growth. Thread.	No Growth. Paper.	Doubtful.	Total.
B. Pyo.	..	2	8	6	..	16
B. Coli	..	4	8	4	..	16

Summary—Total number of tests made and incubated at once, 142.
Total number of tests made and kept 24 hours before incubation, 119.
Of those incubated at once, growth showed in 30; 21 per cent.
Of those incubated at once, no growth present in 112; 79 per cent.
Of those kept 24 hours before incubation, growth showed in 27; 22.6 per cent.
Of those kept 24 hours before incubation, no growth showed in 92; 77 per cent.

Total number of tests made, 261.
Total number of tests showing growth, 57; 21 per cent.
Total number of tests, no growth, 204; 79 per cent.

Test 2.—Formaldehyde, aluminum sulphate and lime method.
Amount of formaline (estimated 40 per cent.), 44 oz. per 1,000 cu. ft.
Range of temperature, 49° C., ½ hour; 55° C. for 2½ hours.
Size of room (steam sterilizer), 276 cu. ft., 3 hours' exposure.
One half tests incubated at once; one-half kept 24 hours before incubation.
Books used: Vol. 1—575 pages, board and linen covers, covers snapped back open.
Vol. 2—288 pages, board and linen covers, covers snapped back open.
Vol. 3—575 pages, board and linen covers, book closed.
Vol. 4—210 pages, board and linen covers, book closed.

Incubated at once:

Books.	Pages.	Cond.	Bact.	Growth. Thread.	Growth. Paper.	No. Growth. Thread.	No. Growth. Paper	Total.
Vol. 1	575	Open	B. Pyo.	..	..	10	10	20
			B. Coli	..	..	10	10	20
2	288	Open	B. Pyo.	..	..	8	8	16
			B. Coli	..	..	8	8	16
3	575	Closed	B. Pyo.	..	..	10	10	20
			B. Coli.	..	1	10	9	20
4	210	Closed	B. Pyo.	..	..	10	10	20
			B. Coli	..	..	10	10	20
				..	1	76	75	152

Summary—Number of tests made and incubated at once, showing growth, 1; 0.4 per cent.
Number of tests made and incubated at once, not showing growth, 152; 99.6 per cent.
Number of tests made and incubated after 24 hours, not showing growth, 132; 100 per cent.
Total number of tests made, not showing growth, 284.
Total number of tests made, showing growth, 1; 3 per cent.
Total number of tests not showing growth, 283; 99.7 per cent.

Test 3.—Formaldehyde and lime and aluminum sulphate method.
Exposure made at room temperature, 24 hours; day clear and dry.
Amount of formaline (estimated 40 per cent.), 18.6 oz. per 1,000 cu. ft.
One-half tests incubated at once; one-half kept 24 hours.
Books used: Vol. 1—290 pages, open.
Vol. 2—358 pages, open.
Vol. 3—352 pages, closed.
Vol. 4—240 pages, closed.

Incubated at once:

Books.	Pages.	Cond.	Bact.	Growth. Thread.	Growth. Paper.	No. Growth. Thread.	No. Growth. Paper	Total.
Vol. 1	290	Closed	B. Pyo.	9	6	3	7	25
2	358	Closed	B. Coli	7	7	..	7	21
			B. Pyo.	11	8	1	4	24
			B. Coli	12	9	1	3	25
3	352	Open	B. Pyo.	6	3	4	7	20
			B. Coli	8	2	2	7	19
4	240	Open	B. Pyo.	6	3	4	7	20
			B. Coli	8	2	2	7	19
				67	40	17	49	173

Summary—Number of tests made and incubated at once, showing growth, 107; 61.84 per cent.
Number of tests made and incubated at once, showing no growth, 66; 38.9 per cent.
Number of tests made and kept 24 hours before incubation, showing growth, 66; 46.52 per cent.
Number of tests made and kept 24 hours before incubation, showing no growth, 53; 54.8 per cent.
Total number of tests made, showing growth, 173; 54.8 per cent.
Total number of tests made, showing no growth, 142; 44.1 per cent.
Total number of tests made, 317; 100 per cent.

Test 4.—Experiment with dry heat.

In this experiment heat alone was used, the books being placed in a steam sterilizer and subjected to dry heat.
The length of exposure was 3 hours.
The temperature varied from 48° to 55° C.
Books used: Vol. 1—Pages 290, book opened, covers held back.
Vol. 2—Pages 358, book opened, covers held back.
Vol. 3—Pages 358, book closed, board and linen covers.

Incubated at once:

Books.	Pages.	Cond.	Bact.	Growth. Thread.	Growth. Paper.	No. Growth. Thread.	No. Growth. Paper	Total.
Vol. 1	290	Open	B. Pyo.	11	14	4	2	31
			B. Coli	14	16	1	..	31
Vol. 2	358	Open	B. Pyo.	2	6	6	2	16
			B. Coli	5	7	3	1	16
Vol. 3	352	Closed	B. Pyo.	3	8	6	1	18
			B. Coli	4	6	5	3	18
				39	57	25	9	130

Summary—Number of tests incubated at once, showing growth, 96; 73.87 per cent.
Number of tests incubated at once showing no growth, 34; 26.5 per cent.
Number of tests kept 24 hours before incubation, showing growth, 46; 61.97 per cent.
Number of tests showing no growth, 37; 20 per cent.
Total number of tests made, showing growth, 169; 68 per cent.
Total number of tests made, showing no growth, 79; 31 per cent.
Entire number of tests made, 248; 100 per cent.
This experiment was repeated with similar results.

Test 5.—In this test the books were exposed to heat alone, the exposure being made in sterilizer and the books subjected to dry heat. Length of time of exposure was 23 hours; temperature ranged from 50° to 55° C.

One-half tests incubated; rest kept 24 hours to determine attenuation.

Books used: Vol. 1—210 pages open, covers held back.
Vol. 2—575 pages.
Vol. 3—575 pages, board and linen covers closed.
Vol. 4—356 pages, board and linen covers closed.

This resulted in death of testing organism, but the books were warped and showed the result of long-continued heating.

Books.	Pages.	Cond.	Bact.	Growth. Thread.	Growth. Paper.	No. Growth. Thread.	No. Growth. Paper	Total.
Vol. 1	210	Opened	B. Pyo.	..	..	8	8	16
			B. Coli	..	..	8	8	16
2	575	Opened	B. Pyo.	..	..	8	8	16
			B. Coli	..	..	8	8	16
3	575	Closed	B. Pyo.	..	..	10	10	20
			B. Coli	..	..	10	10	20
4	356	Closed	B. Pyo.	..	..	12	14	26
			B. Coli	..	..	13	13	26
				..	..	77	79	156

Test 6.—In this test a new book was used to ascertain the action of heat upon the paper and binding. It was exposed for a period of about 2½ hours, at a temperature of 55° C., and beyond a slight spreading of the leaves showed no damage.

ANTIANAPHYLACTIC VACCINATION

Edwin J. Banzhaf, Phar. D., and Edna Steinhardt, M. D.

Considerable work has been done along the lines of preventing anaphylaxis, and of vaccinating without resensitizing.

Among the principal workers in this field in Besredka,[1] who applied experiments on milk anaphylaxis to that of serum. He has found a substance designated by him as "petit sérum," which he compares with the "petit lait," saying it is neither sensitizing nor toxic, but vaccinating.

The method of preparing the "petit sérum" is as follows: Two parts of 90 per cent. alcohol are mixed with one part of serum; the resulting precipitate is treated with physiological salt solution and filtered. The opalescent filtrate is the "petit sérum."

It seemed to us that the "petit sérum" or filtrate, as we will call it, did not contain the vaccinating substance separated from that of the sensitizing and toxic substances, but rather that all his results were due to dilution of one and the same substance. In his most recent article Besredka[2] has also come to the conclusion that the three actions are due to one and the same substance, these actions depending upon its state of coagulation or decoagulation. Before that appeared we had repeated his work.

After obtaining the dilute filtrate according to Besredka's method we concentrated it to about one-fourth of its volume before testing. Physiological salt solution, concentrated to the same degree, was injected into sensitized guinea pigs as controls. These gave no symptoms. One-fourth to one-half cubic centimeter of the concentrated filtrate injected intracardially into serum-sensitized guinea pigs gave in the first set typical symptoms, followed by death.

The work was repeated with a second filtrate, which when concentrated gave us irregular results; out of five serum-sensitized guinea pigs three died with typical symptoms; the other two showed no definite symptoms.

The sensitizing action of the filtrate was also tested. The filtrate was not concentrated. It was given subcutaneously to guinea pigs in doses of one-tenth and one cubic centimeter.

Twenty days later intracerebral injections of one-fourth cubic centimeter normal horse serum were given.

The animals which had previously received one cubic centimeter of the filtrate showed slight symptoms of anaphylaxis, coughing, scratching, diaphragmatic breathing, all slow in appearing. Those guinea pigs treated with one-tenth cubic centimeter filtrate subcutaneously were tested intracerebrally twenty days later. One died with typical symptoms of anaphylaxis. The other two showed slight symptoms, coughing, scratching, diaphragmatic breathing, and weakness of hind legs.

The experiment was repeated with another filtrate, giving the preliminary dose intraperitoneally. Two guinea pigs received one-tenth cubic centimeter of the filtrate. On testing 28 days later, one showed slight but definite symptoms, the other died with typical symptoms. Two guinea pigs received one cubic centimeter of the filtrate. On testing 28 days later one showed slight symptoms; the other was not affected.

In all cases the precipitate, obtained by the addition of alcohol to the whole serum, was thoroughly washed with alcohol to remove any traces of unprecipitated serum. The alcohol was removed from the filtrate by evaporation in vacuo.

TABLE I

Toxic Properties of Besredka's "Petit Serum"

G. P. No.	Previous Treatment	Interval in Days	Second Injection Intracardially	Symptoms	Result	Autopsy.
124.....	.54 cc. toxin-1/400 antitoxic horse serum.	14	¼ cc. concentrated filtrate.	Typical.	Death within 2 minutes.	No hemorrhage in pericardium.
125.....	.54 cc. toxin-1/400 antitoxic horse serum.	14	¼ cc. concentrated filtrate.	"	Death within 2 minutes.	

The above experiment was repeated with a second concentrated filtrate. Three out of five sensitized guinea pigs died with typical symptoms.

TABLE II

Sensitizing Action of Besredka's "Petit Serum"

G. P. No.	Previous Treatment Filtrate	Interval in Days	Second Injection Intracerebrally	Symptoms	Result
335.....	1/10 cc.	20	¼ cc. horse serum.	Typical.	Death within 6 minutes.
260.....	1/10 "	20	¼ cc. horse serum.	Scratching, cough, diaphragmatic breathing.	Recovered.
270.....	1/10 "	20	¼ cc. horse serum.	Scratching, cough, diaphragmatic breathing.	"
271.....	1 "	20	¼ cc. horse serum.	Scratching, cough, diaphragmatic breathing.	"
272.....	1 "	20	¼ cc. horse serum.	Scratching, cough, diaphragmatic breathing.	"

CONCLUSIONS

From our work we consider that by Besredka's method a simple dilution of the active protein has been accomplished and no separation.

However, in all the work done with the end in view of preventing anaphylactic symptoms in man, it has been continually assumed that the reactions occur in man as in guinea pigs.

Why is not the rabbit used as the animal for comparison? Repeated spaced injections of serum or other protein in the rabbit, unless intravenous, do not cause the explosive symptoms witnessed in guinea pigs.

They do cause, if given subcutaneously, an œdematous condition, "the Arthus phenomenon." In man sudden death from a second spaced injection of serum is the great exception, not the rule. The symptoms of serum sickness are not generally grave, and yet the present outlook of to-day is to view man from the standard of the guinea pig and not of the rabbit.

It is true, in the rabbit, typical explosive anaphylactic symptoms are caused by injections of the protein if made intravenously as shown by Arthus,[3] Lesue, and Dreyfus[4] and others. And again, there is another point of difference from the guinea pig. Arthus was unable to produce antianaphylaxis by any of the methods which produce it so rapidly and so readily in the guinea pig.

In the experiments of Lesue and Dreyfus this lack of antianaphylaxis can also be noticed.

We repeated the experiments on rabbits and, although the sensitization was very irregular, we were able to confirm their results.

Therefore, it seems to us that all methods proposed for vaccination against accidents of serum injection, based on guinea pig experimentation, should also be proven on the rabbit before using on man, for man reacts more like the rabbit than like the guinea pig.

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ON THE TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING: A STUDY OF SERUM HÆMOLYSINS IN GOATS*

(Studies on Serum Hæmolysins, II)

L. W. Famulener, M. D.

OUTLINE

I. Introduction: the inheritance problem; its relation to disease, and immunity; fundamental studies; conclusions.

II. General review of work upon the subject of transmission of immunity from mother to offspring.

A. Antibodies of well-differentiated types.
   1. Antitoxins induced by:
      (a) Plant toxalbumins: (1) abrin; (2) ricin; (3) robin.
      (b) Bacterial extracellular toxins: (1) tetanus; (2) diphtheria; (3) pyocyaneus; (4) symptomatic anthrax; (5) vibrio nasik.
      (c) Animal venoms.
      (d) Ferments.
   2. Agglutinins induced by:
      (a) Bacteria: (1) typhoid (clinical observations and animal experimentation); (2) tuberculosis (clinical observations); (3) cholera; (4) proteus.
      (b) Blood: (1) sheep corpuscles.
   3. Precipitins induced by sera.
   4. Cytolysins induced by:
      (a) Bacteria.
      (b) Blood corpuscles.
   5. Opsonins induced by bacteria.
   6. Allergins induced by sera.

B. Antibodies of not well-differentiated types.
   Induced by:
   1. Bacterial intracellular toxins:
      (a) Organisms: (1) anthrax; (2) swine erysipelas; (3) tuberculosis; (4) dysentery.
   2. Spirilla:
      (a) Sp. obermeieri.
      (b) S. pallida.
   3. Protozoa:
      (a) Piroplasmosis (dog).
      (b) Rabies.
   4. Ultramicroscopic organisms:
      (a) Sheep-pox (ovine).
      (b) Foot and mouth disease.
      (c) Smallpox and vaccinia.

   Discussion of results.

III. Report upon additional work concerning transmission of immunity from mother to offspring.

1. Introductory remarks: factors in passive transmission of antibodies; group of so-called intracellular bacterial toxins; immunity against intracellular toxins; complexity of this immunity process; cytolytic bodies as index to serum value; use of hæmolysins in such studies.

* This work was aided by a grant from the Rockefeller Institute for Medical Research.

2. Review of work reported upon transmission of hæmolytic antibodies.
3. Choice of experimental animals and outline of technic.
4. Groups of experiments:
   (a) Active immunization of the adult before birth of the young, either during the period of gestation or before conception had taken place.
      Series I. Preliminary experiments.
      Series II. Special experiments.
   (b) Active immunization of the adult following immediately or shortly after the birth of the young.
      Series III. Animals normal at time of parturition.
      Series IV. Animals partly immunized at time of parturition.
   (c) Active immunization of the newly-born or older kid against foreign blood cells.
      Series V. Blood injections into kids.
   (d) Gastro-intestinal absorption of antibodies.
      Series VI. Feeding of hæmolytic serum to newly-born kids.
5. General discussion of results.
6. Summary.

Bibliography.

I. Introduction.

The inheritance by the progeny of newly acquired biological qualities from either or both parents has been a question of long standing. It has attracted the attention of numerous investigators, particularly physicians, since the beginning of scientific medicine and biology. Certain disease tendencies were considered to be transmissible from parents to offspring (inherited). On the other hand, observations were recorded of cases where the mother, shortly after having recovered from a disease, gave birth to a child, and the child upon exposure to the same disease showed resistance against the infection. As an example of this character may be mentioned variola (Lereboullet, Buchner[1]). Burckhardt[2] made similar observations upon infants born from mothers vaccinated (vaccinia) during late pregnancy. In smallpox an intrauterine infection of the fœtus may occur, in which case not a true inherited but an actively acquired resistance results in the child; possibly the same thing occurs in vaccinia. The transient resistance which infants commonly show against many infections during the first weeks of life, while not fully understood, is probably for the most part of maternal origin.

As is evident, the problem has been beset with difficulties, and very little progress was made toward a solution until the newer discoveries in immunity became known. Immunity actively acquired against specific infectious agents or certain toxins offered itself as a means, readily applicable, by which an insight might be gained concerning the general laws of inheritance, and at the same time afford practical data on the subject of immunity. Following the discovery of the exciting causes of many of the infectious diseases, and the isolation of these specific organisms, animal experimentation was undertaken by several investigators. Chauveau,[3] in 1880, found that by vaccinating with anthrax organisms the slightly susceptible Algerian sheep during gestation their offspring were born immune against this organism. These results were supported later by other workers using symptomatic anthrax (Arloing, Cornevin and Thomas,[4] Kitasato[5]).

A means had now been acquired by which fairly definite tests could be employed under conditions subject to control. More exact methods were introduced for actively immunizing animals against various specific antigens. The resulting degree of immunity became subject to standardization, thus establishing a firmer basis for studying the problem. Subsequently much important work appeared which threw more light upon the question.

The publication in 1892 of the results of Ehrlich's[6] basic studies marked a new epoch in the development of the subject. He carried out immunization experiments by feeding mice the toxalbumins, abrin, ricin, and robin. It was found that the young from immune mothers acquired an immunity, which he considered as passive, that is, a passage over of the specific antibodies to the fœtus before birth, and then through the milk to the nursling after birth. He proved by placing normal young upon immune nurses that the milk transmitted specific antibodies, which were absorbed by the nurslings and caused their immunization. Also, he found that highly immunized males, when crossed with normal females, failed to endow the young with resistant powers against the specific toxin, so concluded that the idioplasm of the sperm did not carry over immunity to offspring.

In opposition to Ehrlich's assertion that the immunized male alone was unable to transmit an acquired immunity to his descendants, several workers brought forward experiments which conflicted with his results. Among these workers may be mentioned Gley and Charrin,[7] Tizzoni and Cattani,[8] and Tizzoni and Centanni.[9] Ehrlich and Hübener[10] (1894) repeated similar experiments, using tetanus toxin against both guinea pigs and mice, with results which sustained Ehrlich's first findings. In a critical review of the opposing work they pointed out certain experimental conditions in each case which they considered invalidated the results. The investigations of Wernicke[11] (1895), Vaillard[12] (1896), and others (Remlinger,[13] [65]Dieudonné,[14] Bulloch,[15] etc.) fully supported Ehrlich's findings. So at the present time the view, for the most part, is generally accepted that acquired immunity is not inherited by the offspring in the ontogenetic sense. In the mammalia apparently the germ plasm of neither parent plays an appreciable rôle in transmitting an acquired immunity to young.*

In discussing the question of the transmission of an acquired immunity from parents to the offspring, attention must be directed to the mother. The specific immunity of the offspring is a passive condition, and not a biological inherited condition of long standing. Therefore, the subject may be transferred from the province of biological heredity (germplasm) to the field of practical medicine and prophylaxis, and be considered from that standpoint.

II. General review of work upon the subject of transmission of immunity from mother to offspring.

An extensive literature pertaining to the transmission of immunity from mother to offspring has developed in the past two decades. Since we have had occasion to make a rather exhaustive review of this literature, perhaps it may be of interest if a brief outline of the more important papers dealing with the subject be submitted. Special consideration will be given those papers which are important either from a historical standpoint (positive results), or those of fundamental value in the development of the subject. No attempt will be made to even briefly abstract the numerous articles, but, instead, a provisional classification of antibodies and antigens will be outlined, and under these headings the references will be catalogued. These papers, in general, concern the transmission of specific antibodies to the young by actively immunized animals. In some instances simply the presence of antibodies in the milk of such actively immunized animals is reported. Purposely the work upon passive immunization of the mother animal is omitted, since in our studies attention is directed only to actively immunized mothers and their offspring.

*It may be of interest to mention in this connection the work of Klemperer (Arch. f. exper. Path. u. Pharmacol., 1893, XXXI, 356) who showed that the yolk of eggs from hens which had been actively immunized against tetanus, showed antitoxin. Dzierzgowski (Ref.-Centralbl. f. Allg. Path. u. path. Anat., 1901, XII, 715) confirmed these results with hens actively immunized against diphtheria. He states that chicks hatched from such eggs showed diphtheria antitoxin in their blood serum.

A. Antibodies of well-differentiated types.

1. Antitoxins.—Animal experimentation has been carried out upon the following sub-groups of toxins: (a) Plant toxalbumins.—As mentioned earlier, animals immunized against abrin, ricin, and robin transmitted the antibodies to their own young, or to normal sucklings (Ehrlich[6]). (b) Bacterial extracellular toxins: (1) Tetanus.—Actively immunized rats and rabbits (Tizzoni and Cattani[16]), guinea pigs and mice (Ehrlich and Hübener[10]) and mare (Ransom[17]) transmitted an immunity to their offspring. It is of historical interest that Brieger and Ehrlich,[18] in a study upon antitoxin-bearing milk from a goat immunized against tetanus, published the first immunity curves. (2) Diphtheria.—In diphtheria clinical cases have been reported in which mothers recovering from the disease have apparently transmitted antitoxins to their infants (Kayser[19] and others). But it has been shown that a high percentage of infants from normal mothers show antitoxic properties in their sera after birth; therefore, the interpretation of the above observations is open to question (Fischl and von Wunscheim[20]). Among animal observations guinea pigs (Wernecke,[11] Anderson[21]) and mare (Salomonsen and Madsen[22]) have been shown to transmit immunity or antibodies against diphtheria to their young. (3) Pyocyaneus.—In some cases rabbits (Charrin and Gley[23]), when immunized during the course of pregnancy, transmitted, to a certain degree, specific protective powers to their young. (4) Symptomatic anthrax.—As mentioned, different workers have found that a certain degree of immunity is transmitted from the recently immunized mother to her young (Arloing, Cornevin and Thomas,[4] Kitasato[5]). (5) Vibrio Nasik (vibriolysin).—Wegelius[24] reports that goats and rabbits, when immunized either shortly before conception or during gestation, transmit the specific antibodies to their young. (c) Animal venoms.—Frazer[25] states that a cat which was immunized against snake venom during the course of pregnancy gave birth to young which suckled mother; one kitten, when given two miminum lethal doses (subcutaneously) on the fifty-seventh day, showed marked resistance against the venom with only very slight symptoms, from which it soon recovered; the other kitten on the sixty-ninth day was given three fatal doses of venom, from which it failed to survive. (d) Ferments.—Morgenroth[26] immunized goats against rennin and found in a study of the milk that the specific antibody was present in considerable amounts. No example was found in the literature where studies were made upon the transmissibility of antibodies of this type from mother to young.

2. Agglutinins, including (a) bacterial and (b) hæmagglutinins. (a) Bacterial agglutinins: (1) Typhoid.—In view of the fact that much work has been done upon this group of agglutinins both (a) clinically (patients) and (b) experimentally (animals), a brief outline of the two sub-groups will be given. (a) Among the clinical observations of possible intrauterine transmission of typhoid agglutinins from mother to fœtus a number of cases have been reported in which the mother aborted during the course of the disease. The body fluids from fœtuses ranging in age from three to six months failed to show specific agglutinins (Etienne,[27] Charrier and Apert,[28] Dogliotti[29]). However, Scholtz[30] reported a strong agglutinating reaction from fluids of a seven months' fœtus. In other cases (Schumacher,[31] Stäubli,[32] Mosse and Daunic[33]) blood taken from the fœtal end of cord at birth (about or full term) of children born from mothers who had suffered during the course of pregnancy from typhoid gave positive reactions. Other cases are reported (Castaigne,[34] Courmont and Cade[35]) in which infants taking the milk from mothers who were infected as late as two months (one case) after having given birth to child also showed positive agglutinating sera. However, this was not always the case, since in a few instances (Kasel and Mann[36]) the milks (2) were positive (1:25)—the blood agglutinated 1:50—but no reaction was given by the suckling's sera. The mothers in this series were infected two to twenty-one years before parturition. In another instance (Achard

and Bensaude[37]) a mother contracted the disease while nursing the child (age ?); the milk showed a low agglutinating value (1:10); the blood of the nursling was negative. In the other cases the milk from the mother, when tested, showed a positive reaction with few exceptions. (b) The published studies upon animal experimentation add nothing further to the results already cited. Widal and Sicard[38] reported positive results in young rabbits born from a mother which was inoculated with the typhoid bacillus six days before parturition. The heart blood taken from young at time of birth, however, was much weaker in agglutinating power than the mother's blood. Guinea pigs actively immunized during pregnancy in most cases transmitted agglutinins to their young (Remlinger,[38] Jurewitsch,[39] Stäubli[40]). It is noteworthy that a goat which was highly immunized late in pregnancy gave birth to a kid whose blood showed no specific agglutinins (Schumacher[31]). This kid had not suckled before a blood sample was taken.

Among other organisms whose specific agglutinins have been reported as passing to the young from the actively immunized mothers, the following may be mentioned: (2) B. Tuberculosis;* specific agglutinins passed from infected mother to child (Lagriffoul and Pages[41]). (3) Cholera.—Specific agglutinins were transmitted from guinea pigs, immunized during pregnancy, to the fœtus or the young (Achard,[42] Dieudonné[14]). (4) Proteus.—Specific agglutinins were found in the serum of young guinea pigs from immunized mothers (Achard[42]).

Specific agglutinins for the above and other micro-organisms have been demonstrated in the milk of immunized experimental animals. But, as is evident, it does not follow that agglutinins are transmitted with any regularity from mother, either intrauterine or through the milk, to the offspring. For instance, a goat showing a reaction of 1:400 against anthrax failed to pass much agglutinin over to the still-born young; the kids' sera reacted in 1:10 dilution, which was much less than was normally present in the mother's blood before inoculation (Gengou[43]). (b) Hæmagglutinins.—Kraus[44] reported that transmission of immune hæmagglutinins did not take place in a kid born from a mother which had been immunized against red blood cells of sheep, but these immune bodies were demonstrated in the goat's milk. In infants' blood serum (cord) at time of birth natural hæmagglutinins were found to be less in amount than in the mother's serum (Halban,[45] Halban and Landsteiner[46]), although there was much variation, depending upon the source of the test corpuscles.

(3) Precipitins.—Specific precipitins against human blood serum have been reported by Merkel[47] in the serum of young rabbits (blood taken shortly after birth) from mothers which were immunized during the period of gestation. He considers that it was a case of placental transmission. Bertarelli[48] immunized two dogs by subcutaneous injections of horse serum and of cow serum respectively; later he found a small amount of precipitins in the milk of each, but found none in the blood serum of their young after four weeks' nursing.

4. Cytolysins: (a) Bacteriolysins.—Normal bacteriolytic bodies have been frequently demonstrated in different milks, likewise specific bacteriolytic substances have been demonstrated in the milk of immunized animals (Kraus[49]). No experimental work has been found demonstrating a transmission of specific bacteriolysins from the mother to the young. Clinically several investigators have reported the relative bacteriolytic values of the mother's and infant's serum at the time of birth (Halban and Landsteiner[46]); the infant's serum showing a less amount of natural bacteriolysins than the mother's serum. (b) Hæmolysins.—Several investigators have reported upon the transmission of specific hæmolysins from immunized mothers to offspring, either through the placenta or the milk. Immunized rabbits appear to transmit the hæmolysins to the fœtus through

*Figari (Centralbl. f. Bakteriol., I. Ref., 1906–7, XXXIX, 75) reports that hens fed upon tubercle bacillus preparations, after a time showed specific agglutinins in their eggs.

the placenta (Bulloch,[15] Bertino[50]). Goats may or may not (Kraus,[41] Kreidl and Mandl[51]). In one case sheep failed to do so (Bertarelli[48]). Natural hæmolysins have been reported as being present in some cases in the serum of infants at birth. The question of the transmission of hæmolysins will be taken up later in this paper and be fully considered. (5) Opsonins.—Turton and Appleton[52] compared the opsonic index of mothers' and infants' sera in two cases (infants' ages, four and seven days) and found the infants' sera much lower than that of the mothers. Also they found in comparative tests between the mother's serum and milk that the latter was very much weaker in opsonins. It was not known whether the infants absorbed opsonin from the milk or not. This work has been in the main supported by other observers, both in human cases and by animal experimentation (von Eisler and Sohma[53]).

6. Allergin.—It has been shown that female guinea pigs which have been sensitized by injections of proteins, as horse serum, bear young which are also hypersensitive to the same serum during the early weeks of life (Rosenau and Anderson,[54] Anderson,[55] Gay and Southard[56]).

B. Antibodies of not well differentated types. Induced by:

1. Bacterial Intracellular Toxins.—As is well known, a large group of pathogenic bacteria do not excrete specific, soluble toxins to any extent; so their poisonous properties are supposed to be mostly due to cellular proteids, or so-called intracellular toxins. Active immunity may be developed by recovery from the disease, or by experimental vaccination with killed or attenuated cultures. Not much is known concerning this type of immunity, but it is probably quite complex in nature and dependent upon a number of factors. However, it has been experimentally proved that a mother immunized with certain members of this group may transmit a specific immunity to her young, for example: (1) anthrax (Chauveau[8]); (2) swine erysipelas (Ehrlich[6]); or, (3) tuberculosis. Behring[57] observed that a fourteen-day-old calf born from a highly immunized cow (bovine tuberculosis) possessed considerable immunity against the organism. Behring thought that this immunity was acquired by the calf through the milk, rather than by intrauterine transmission. He suggested the milk of specifically immunized cows as a means of combating tuberculosis in man, either as a preventive or a therapeutic measure. (4) Dysentery.—De Blasi[58] actively immunized a rabbit against the dysentery bacillus by three inoculations of killed cultures during the first 18 days after birth of young. The young (2) were given on the 19th day surely fatal doses of the dysentery culture. Both remained alive, whereas controls died.

2. Spirilla: (a) Sp. obermeieri.—Animals may be actively immunized against this organism by further injections of spirillar blood following a recovery from the infection; others may be passively immunized by the injections of serum from such animals. Novy and Knapp[59] found young rats born from infected mothers immune when tested after a few weeks. They looked upon this as an active immunity, due to intrauterine infection, since the spirilla may penetrate the placenta and reach the fœtus. Therefore, it remains an open question if an active immunity (intrauterine infection) or passive immunity (placenta, milk) from mothers' antibodies occurs in the young. (b) Spirochæta pallida (syphilis).—It is questionable whether a complete immunity ever follows in syphilitic patients after a clinical recovery from the disease. With the introduction of the complement fixation test in the diagnosis of syphilis, it has been found that many apparently healthy children born from luetic mothers give a positive reaction, while others, non-syphilitic, may contract the disease by suckling a diseased mother (Ricketts and Dick[60]). This would prove Profeta's Law * can no longer hold. It would appear that whatever immunity exists is slight. According to Thomsen,[61] a number of observers besides himself have found complement-fixing substances in the milk from luetic women. It would be of interest to know if an infant taking

*Profeta's Law assumed that a healthy child might be born from a luetic mother, and suckle the mother without contracting the disease.

such milk from a luetic mother would absorb these bodies in sufficient quantity to give a positive reacting serum. It is still questionable whether these complement-fixing bodies are true antibodies, or simply metabolic products brought about by pathological changes in the body of the patient during the course of the disease.

3. Protozoa: (a) Piroplasmosis (dog).—Kleine and Möller [62] showed that immunized dogs transmit some immunity to their young against this disease. (b) Rabies.—This disease is only tentatively placed under this division, since the question of the protozoan nature of the so-called Negri bodies is still under discussion. Hoegyes [63] was one of the first workers to point out that actively immunized female dogs may transmit some immunity to their offspring. Konradi [64] carried out similar experiments upon dogs and in several cases with positive results. The work of Remlinger [65] with rabbits also supported those results.

4. Ultramicroscopic Organisms.—Under this heading may be placed a group of contagious diseases which, on account of the constant failure to demonstrate definite specific micro-organisms, has led many workers to consider the contagion in each case to be ultramicroscopic in size. (a) Sheep-pox ("ovine").—According to Burckhardt,[2] Richert immunized a large herd of sheep against ovine during the last six weeks of gestation. The lambs of these mothers were inoculated with ovine-lymph at the age of four to six weeks; none of them contracted the disease. A large number of controls showed pustules. (b) Foot and mouth disease.—Löffler [66] observed a calf born from a cow immunized against this disease which proved to be immune against inoculations of the virus. (c) Smallpox and vaccinia.—Since in the introductory portion of this paper smallpox has been briefly but sufficiently discussed, it will not be considered further. The work of Burckhardt [2] upon smallpox vaccine is of special interest, since it was one of the first investigations upon the possible transfer of protective substances from mother to the child in utero. He vaccinated mothers during the last weeks of pregnancy, and then repeated the vaccinations upon the infants during the first days of their lives. He found in several that an apparent protection against the vaccinia had been acquired. Most of the control infants gave positive reactions. While these results were not conclusive, they offered suggestive data. In this connection it may prove of interest to note that in 1799 Hufeland (cited by Neumann [67]) was perhaps the first to suggest that the milk might exert a protective power for the child when secreted by a mother who had recently recovered from the disease. In this class of disease, as has been previously pointed out, it is always a question whether the young have been passively immunized from the mother's antibodies, or if an active immunization has taken place from having had the disease before birth.

DISCUSSION OF RESULTS

In reviewing the literature on this subject one is impressed by the broad scope of the studies, and the great amount of work which has been done. Even in the brief review just given it is evident that the work on active immunity has been very productive of positive results. Also much work has been done by passively immunizing pregnant and nursing animals, but this has been purposely omitted in our résumé, since our studies cover only actively immunized mothers as influencing the offspring.

The possibility of an active intrauterine immunization on the part of the fœtus must always be considered in connection with this type of experiment. Such may occur either through the passage of the living infectious agent or its products from the mother to the fœtus. A distinction must be made between this condition and a passive immunization by means of the mother's antibodies through the placenta or through the milk to the young. In the diseases which are supposed to be due to ultramicroscopic organisms the virus not infrequently seems to be able to pass the placenta and affect the fœtus in utero. Where immune off-

spring are born from mothers which have passed through such a disease during pregnancy, caution must be exercised against accepting this as a true passive transmission of immunity (in utero) from the mother. To this we must add that animal experiments with certain living spirilla must be considered in the same light, since they may also pass the placenta and affect the fœtus.

Most bacteria do not pass the intact placenta, except under extraordinary conditions. Further, recent work (besides our own) would indicate that the fœtus, or the very young animal, possesses only feeble reactive powers as to antibody production when attempts have been made to actively immunize by means of prepared antigens. As shown by the foregoing review, fluids from fœtuses aborted early in pregnancy by infected mothers usually gave negative results when tested for specific antibodies. Judging from these investigations, we should expect the best results in the young from experimental animals which have been actively immunized either against dead cells or toxins during the latter part of gestation. Even where such conditions were observed, positive results did not follow in every case. In part the negative results may be accounted for by differences in experimental conditions, such as nature of antigen used, degree of active immunization attained, time of immunization relative to birth of young, character of resulting antibody, time when blood from offspring was first taken, etc. Further, many workers failed to make any distinction between the rôle played by placental or by milk transmission of antibodies; in many cases the blood of the young was not drawn until after having the mother's colostrum and milk. In fact, blood samples in some instances were not taken until weeks after the birth of the young. Much of the literature cited in the above review covers very careful and painstaking investigations which have been carried out upon this subject. These studies prove that when the mother has been properly immunized during gestation and shows a high degree of immunity at the time of birth of young some of this immunity (antibodies) may be passively transmitted to her young. This occurs either through the placenta before birth, or by means of the milk immediately after birth.

III. Report on Additional Work Concerning Transmission of Immunity from Mother to Offspring.

1. Introductory Remarks.—As pointed out in the foregoing résumé, the passive transmission of antibodies to the offspring is practically established. This seems to hold for almost all classes of infectious organisms where a true active immunity (formation of antibodies) takes place in the mother's body. But the relative importance of the exact parts played by the two separate means of transmission—placenta or milk—has not been so fully decided. Some workers maintain that the placenta plays the greater rôle, while another, but smaller, group holds that antibodies do not pass the intact placenta. More work upon this subject, bearing in mind this phase of the question, seems desirable. A large group of infectious diseases is induced by organisms which do not produce extracellular toxins (in culture) sufficient in amount or of such a nature that antitoxins can be produced by animal immunization. Recognizing the importance of this group of endotoxin-producing organisms and their significance in disease, it was thought worth while to take up analogous studies which might throw more light upon the transmission of immunity to offspring. Since if by vaccinating the mother cautiously during pregnancy the offspring also becomes immunized, a means is offered by which the young may be protected against those infections (pneumococcus, etc.) to which they are particularly susceptible during the first weeks of life. Up to the present time we have no curative serum which offers much hope in these conditions. Active immunization (by vaccines) of the newly-born against these organisms offers little encouragement, and, in fact, may be followed by harmful sequelæ. Not much is known concerning the nature of the immunity induced by actively immunizing animals against this type of organism. But, perhaps, in so far as the serum of such animals is concerned, the contained antibodies are quite complex in

nature, and include antitoxins, bacteriolysins, opsonins, etc. Also there seems to be evidence that such a serum contains substances of which we know little, other than that they produce favorable results, exclusive of the bodies already mentioned. Since cytolysis must be one of the important factors in this type of immunity, the cytolysins may act as an index in carrying out experimental studies upon such immune sera.

As mentioned in the review, little has been done directly upon the bacteriolytic antibodies concerning their transmission from mother to offspring, but both natural and specific bacteriolysins have been found in milk from different animals. Most workers who have carried out studies along cytolytic lines have used lysins against red blood cells—the hæmolysins.

In my own work, which is to follow, this means was adopted, as it appeared most advantageous. For instance, the test can be carried out completely in test tubes, and, under proper technic, with a high degree of accuracy. Also test animals, as necessary in diphtheria or tetanus antitoxin work, can be entirely eliminated. As compared with bacteriolytic work the hæmolytic tests are much more rapidly performed and probably more easily graded.

2. Review of Work Reported upon Transmission of Hæmolytic Antibodies.—After a careful search through the literature I find that several investigators have already reported work concerning the transmission of hæmolysins from mother to offspring. However, some of this work escaped my attention until after my own work was well under way. Although briefly mentioned in the previous general review, these reports will now be considered more fully.

Kraus [44] in 1901 reported his work upon the possible transmission of immune hæmagglutinins and immune hæmolysins from mother to the offspring. He found that rabbits immunized by repeated subcutaneous injections of defibrinated dog's blood showed in their milk specific hæmagglutinins, but these bodies were not carried over to the blood of the suckling. For the study of hæmolysins he immunized goats by injecting with sheep blood. No hæmolysin could be found in the milk, so he concluded that these immune bodies were not excreted by the mammary glands, although the blood serum of the same animal showed quite definite amounts of the hæmolysin. However, he found that the young born from one of the immunized goats showed a low degree of immunity (hæmolysin) which was transient. Kraus thought that this was acquired before birth, and considered it a passive immunization from an actively immunized mother. He gives no definite data concerning the hæmolytic tests of the serum, but apparently he added no normal complementing serum, but depended upon the small amount of complement which was present in the immune serum used in the test. As is well known, goat's serum is weak in hæmolytic complement, and therefore will not bring out the highest efficiency of the contained immune hæmolysin, even when present in large amounts. As a result his data show the hæmolytic sera to be of very low value. But in the milk tests he added normal goat serum, as complement, since he thought it possible that the milk glands might not let the complement through; consequently no activation of the immune body could take place in case it were present. In view of negative results, he concluded that immune body was not present in the milk. He did other experiments with goat's milk which indicated that it was deficient in complement.

Following this, Bulloch [15] in 1902 took up the study of transmission of hæmolysin from parent to offspring, using rabbits immunized against blood cells of the ox. He considered not only the rôle of the immunized mother, but also that of the father, from the standpoint of heredity, and obtained results which, on the whole, supported the early work of Ehrlich. But of special interest to us are his findings concerning the transmission of specific hæmolysin from mother to the young. When the blood injections were given to the gravid animal pregnancy was interrupted, but the fœtal fluids were found strongly hæmolytic. In those cases in

which the injections were made prior to conception the young were reared, and in one case the inactivated serum of the young was equal to that of the mother in hæmolytic power, while the milk was only one-third as active as the mother's serum. In another case the serum of the young, which was found dead on the day of birth, was one-half the value of that of the mother, while the milk was about one-fourth of that value. Bulloch agreed with Kraus that no complement passed out with the milk,* but showed in opposition to Kraus that hæmolytic immune body is excreted by the milk glands. He used fresh guinea pig serum as complement in all of his tests.

Kreidl and Mandl[51] (1904), in a preliminary report, stated that goats injected with beef blood might lead to a passive immunization of the fœtus, but from their tests concluded that in the majority of cases the specific hæmolysins do not pass from mother to fœtus through the placenta. They considered individual variations to be responsible for the lack of uniformity in their results. No definite experimental data are given in this report. They also consider in this report the transmission of immune hæmolysins from the fœtus over to the mother, but, as their results are not fully conclusive, they will not be discussed in this connection.

Bertino[50] (1905) states that in rabbits, when the immunization of mother has taken place before conception, the lysins do not pass over to the fœtus, nor to the young during the suckling period, but when the immunization is carried out during pregnancy there is a transmission of the specific antibodies to the fœtus. Since the original article was not available, these results are quoted from an abstract (Folia Hæmatologica, 1905, II, 624), and I am unable to give further details.

Bertarelli[48] (1906) reports that he found a small amount of specific hæmolysins in the milk of a sheep immunized against hen's blood. But found none in the blood of the young. This immunization was carried out by repeated injections of the blood during the last weeks of pregnancy and the first weeks after birth of lambs.

Considering the report just presented, it would appear that hæmolysins may be transmitted through the placenta to the fœtus in utero, especially in the examples cited by Bulloch. But objections may be raised as to the validity of the findings from aborted fœtuses, since in those cases a placental lesion could exist which would permit a direct connection between the maternal and fœtal circulation. Apparently the ox blood injected seemed to exert a toxic action upon rabbits. It would be difficult to exclude the influence of absorptions of hæmolysins from milk previous to time blood samples were taken from young in some of the cases. The question of animal species may enter largely in determining the results. The meager data available in Bertino's work upon rabbits would support the statement of Bulloch. Perhaps larger animals, as goats or sheep, do not permit this class of antibodies to pass the placenta so readily as some of the smaller animals. Another important point which must be considered in such experiments involving specific hæmolysins is the presence of natural hæmolysins (for the blood used) in the blood serum of the mother, and their development in the body of the fœtus, or possible passage over from the mother's circulation. Much variation in this respect may exist between different individuals of the same species. It is necessary carefully to control adult's serum as to content of natural immune bodies against the particular blood to be used for immunizing purposes before any injections are made.

3. Choice of Experimental Animals and Outline of Technic.—In the present work goats were selected for experimental animals, since they are more highly developed, rugged, and withstand experimental handling

*Lane-Claypon (Jour. Path. and Bacteriol., 1908-09, XIII, 34) claims to have demonstrated complement in fresh milk (in about 1-10 value of the individual's serum) as well as natural immune bodies, by the addition of the "ox colloid" of Bordet. Others have also shown the presence of the haemolytic complement in milk (Pfaundler u. Moro; Ztschr. f. exper. Path. u. Therap., 1907, IV, 451).

(injections, bleedings, etc.) very readily, and usually give a bountiful supply of milk. Sheep corpuscles were used for immunizing purposes, their advantage being that goat serum seldom shows the presence of more than a small content of natural hæmolysins, if any, against these corpuscles. Goats readily respond to the blood injections by specific hæmolysin production. This immunity may be raised to a high degree by repeated injections of the blood, while practically no toxic action is exerted upon the animal. Moreover, only a small amount of hæmagglutinins are formed by goats against sheep corpuscles; hæmagglutinins, when present in large amounts in a hæmolytic serum, cause a marked disturbance in the hæmolytic reaction. In my experiments the technic followed was similar in all cases and may be briefly outlined as follows:

1. Preparation of sheep's blood for injection or for hæmolytic test: Blood was drawn from the jugular vein of normal sheep by puncturing the skin and vessel wall with a large hollow needle and collecting the flow in a sterile bottle containing a coil of wire. It was defibrinated by shaking, then placed in tubes, centrifuged, the serum removed, and then the corpuscles were washed three to four times in 0.9 per cent. NaCl sol. until they were practically free from all serum.

2. The injections of blood in these experiments were all made subcutaneously. If the volume was very large, it was divided and injected into the animal in several places.

3. Collections of blood samples: Bleedings were made from the jugular vein. In case of adult a hollow needle was used, but from kids a few cubic centimeters were drawn by means of a hypodermic syringe. In each case blood was placed in a test tube and allowed to clot out. When the serum had separated it was removed and placed in small sterile bottles.

4. Milk samples had 10 per cent. volume of 1:1,000 formaldehyde in 0.9 per cent. NaCl solution added. Preliminary tests showed that this strength of formaldehyde solution exerted an antiseptic action, while it had little, if any, influence upon hæmolytic antibodies in milk. The milk samples were then placed in bottles for storage. Both serum and milk samples were stored in ice box, where they were kept until the entire series covering the experiment were collected. Tests were done with the fluid portion which was removed from under the cream layer, after this had separated. The first colostrum, which was heavy and tenacious, failed to separate a cream layer, so was used as a whole.

5. When a full series of samples of sera and milks for a given experiment were collected, all were tested at the same time, thereby using the same blood suspension, complement, technic, etc., which made the results much more comparable. Preliminary tests were first made to get relative values of the samples. The serum became "inactivated" by aging; no heat was applied in any case. The ordinary test tube method for the determination of specific hæmolytic immune body* was employed.

For complement in the earlier experiments, fresh rabbit serum (0.20 c. c. per tube) was used; later fresh serum from guinea pigs (0.10 c. c. per tube) was used. This proved to be more efficient, and was used in all tests which are cited except when otherwise noted.

The scheme followed for the tests was to arrange a series of test tubes for each sample; the serum or milk which was to be tested was first accurately measured in decreasing amounts directly from sample or from the proper dilution in physiological (0.9 per cent.) salt solution dilution, into a series of test-tubes. Then physiological salt solution was added to bring the volume of each tube up to 1.0 c. c. To this was added the given volume of complementing serum, then 2 c. c. of 7½ per cent. suspension of sheep corpuscles. Immediately the tubes with the mixtures were thoroughly shaken, then placed in the incubator (36°-

*The term "hæmolytic immune body" is used in this report as a more suitable equivalent than "substance sensibilisatrice" (Bordet), or "intermediary body," "amboceptor" (Ehrlich) for the biological reaction product of the organism against injected alien blood cells.

37° C.). After one hour they were removed, again thoroughly shaken and placed in the ice-box until any remaining intact corpuscles settled to the bottom of the test tube. After the corpuscles had sedimented a transparent fluid remained above, varying from a deep red color to a water white, depending upon the presence or absence of hæmolysis. The results of the tests were read by making equi-colorimetric readings throughout the various series of samples by comparing with a "standard color" tube which was made by "laking" a given volume of the blood corpuscles in distilled water.

4. Groups of Experiments.

For convenience the experiments will be considered under four groups; the first two cover the main tests upon the transmission of hæmolysins from the mother to the young, while the remaining groups will cover certain tests which have a general bearing upon the subject.

In the first group the female goats were actively immunized against sheep corpuscles by the method outlined. Each experiment was a comparison of the relative content of specific hæmolysins in the mother's serum, the serum of her own young, and her colostrum or milk. No "cross nursing or feeding" of young was undertaken. The tests are not given in perfect chronological order, but each experiment will be given a number for reference purposes, and these will run consecutively throughout the report. In order to avoid repetition, typical experiments will be reported in full; others failing under the same head will be briefly described.

(a) Active immunization of the adult before birth of the young, either during the period of gestation, or before conception had taken place.

Series 1. *Preliminary experiments.* To obtain data concerning the possible transmission of hæmolysins from mother goats to their young, preliminary experiments were undertaken. Since the results proved instructive from several standpoints, a part of the data will be submitted. In this series pregnant animals were immunized during the last weeks of gestation or immediately before the birth of the young. Blood samples were taken from the adults before the injections were started, and then at short intervals during the course of immunization. On day of birth of the young blood samples from the mother and young, and a sample of the mother's colostrum, were taken. Following this, samples were taken, at intervals, for 2½ to 5 weeks or even longer.

Exp. 1. Goat No. 4. (Normal; pregnant.)

During the course of nine days this animal received six subcutaneous injections (increasing doses) of washed sheep corpuscles—a total of about

Time	Mother's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young.....	0.06 c.cm.*	0.02–0.03 c.cm.	0.035–0.04 c.cm.	0.045 c.cm.
1st day after birth.....	.....	0.20–0.30 "	0.04 "	0.05 "
2nd " " "	0.055 "	Traces	0.045 "	0.08 "
4th " " "	0.06 "	0	0.06 "	0.08 "
6th " " "	0.035 "	0	0.08 "	0.09 "
10th " " "	0.047 "	0.20 c.cm.	0.10 "	0.175 "
14th " " "	0.075 "	0	0.15 "	0.20 "

*When comparisons are made between the samples it must be understood that the potency value is inverse to the quantity value. The numbers indicate the volume of each sample which was required to produce a given degree (constant) hæmolysis in that test.

400 c. c. On the morning of the twelfth day after the last injection it was found that she had given birth to two normal kids; one was removed from the mother about noon and placed on cow's milk, although it had undoubtedly freely suckled its mother. Late in the afternoon blood samples were taken from each kid. During the same forenoon both blood and colostrum milk samples had been taken from the mother. Test samples from each were taken at intervals for over two weeks following the birth of the young. Comparative tests (regular technic, 0.20 c. c. rabbit serum for complement) were made upon all of the samples at the same time, and the above results were given.

Further tests showed that the antibody content of the blood in both kids continued to fall, more or less, in parallel. The adult's serum showed its highest value on the sixth day after the birth of the young (eighteenth day after the last injection of blood), then gradually declined. The milk showed transiently (eighth-tenth day) demonstrable amounts of antibodies, following shortly after the adult serum showed its greatest concentration in antibodies.

Exp. II. Goat No. 14. (Normal; pregnant.)

Two subcutaneous injections of washed sheep corpuscles were given on consecutive days: first day 125 c. c.; second day about 140 c. c. Twenty-one days after the last injection the mother was found in the morning with one normal kid, which was allowed to remain with her. At noon blood samples were taken from both, and also a sample of the mother's colostrum-milk. The kid had suckled and stomach seemed full at time the bleeding was made. During the course of immunization blood samples were collected from the mother, and this was continued, with the addition of milk samples at intervals for some time after parturition. Likewise blood samples were taken from the kid, and finally all samples were tested at the same time to get the relative hæmolytic values. The adult's serum showed its maximum hæmolytic value (0.0075 c. c.) thirteen days before birth of young. From this high value it rapidly receded until on the day of birth of young it gave a comparative value of only 0.035 c. c. It remained fairly constant at the same level for about one week, then gradually fell. The colostrum milk showed a value of 0.05-0.06 c. c. on day of birth of young; the antibodies rapidly disappeared from the milk so that by the second day after the birth the test failed to show their presence. The kid's serum, from blood sample taken on day of birth, showed a value of 0.017 c. c., while a sample taken a few days later showed about twice this value, then gradually receded. This would lead us to believe that gastro-intestinal absorption of hæmolysins from the milk is an important factor in the very young animal. The first blood sample was taken from the kid a few hours after birth, and although it had freely fed the absorption of antibodies was still incomplete, but the assimilation from the digestive tract continued after this time, as was indicated by higher value of its blood serum taken later. This question will be taken up again and more fully discussed.

Exp. III. Goat No. 11. (Normal; pregnant.)

This animal was given subcutaneous injections of washed sheep corpuscles spaced three days apart—three in all, 100 c. c., 75 c. c. and 75 c. c. A kid was born during the evening or night following the last injection; it was allowed to remain with the mother. The mother's serum showed no definite immune bodies on day of birth of the kid. Two days later blood samples from both were drawn and a milk sample was taken from the mother; this was repeated on alternate days for several weeks. All were tested at the same time, using 0.20 c. c. of rabbit's serum for complement.

The mother's serum showed its highest value in specific hæmolysins on the tenth day after last injection of blood cells, then gradually receded from this point. The milk showed a low antibody content; the mother's serum and milk reached their highest concentration simultaneously

(serum 0.0035 c. c., milk 0.20-0.25 c. c.). The kid's serum showed questionable traces of specific hæmolysin on the fourth and sixth days after birth, but none later, although the blood serum was watched for several weeks. This test shows that an immunity to erythrocytes arising during the period of lactation has an influence upon the milk, but that the antibody content is less than 2 per cent. of that of blood. Further, a kid at this age must receive much higher quantities of antibodies through the milk, in order to absorb a sufficient quantity to be easily demonstrable in its blood serum. This will be more fully supported by results of other tests which are to follow.

These preliminary tests indicate that mothers actively immunized against the erythrocytes during the latter part of pregnancy can transmit these antibodies to the offspring. But when the immunization has progressed only slightly, that is, no antibodies are demonstrable in the blood until after the birth of the young, the young fail to show more than a trace of antibodies in their blood. Presumably these are acquired through the milk. Probably in all cases this transmission was passive, and took place either through the placenta or the colostrum-milk, or both. I was led to think from these early tests that of the two factors each was about equal in importance. It was found that the colostrum-milk was rich in specific antibodies when the females were immunized before parturition. Also, the kids showed a rise of antibody content in their blood as they took and absorbed this colostrum, which rapidly reached a maximum and then decreased slowly as in passive immunization. Further, it was observed that an animal which received the first blood injection only a few days before and the last on day of birth of young did not influence the offspring to any appreciable extent; the milk showed a very low antibody content.

Series II. Special Experiments.

A series of experiments were undertaken by which the relative importance of the two factors—placenta and colostrum-milk—in the transmission of hæmolysins to the young could be determined. For this purpose goats were actively immunized against sheep erythrocytes in the regular manner, either during the course of gestation, or before conception had taken place. Blood samples were taken from each goat before the injections were started and at short intervals during the course of immunization.

To exclude the possibility of the young getting any milk immediately following birth, the ends of the mother's nipples were previously sealed with collodion. After birth of the young blood samples were taken; the collodion seals were removed from the mother's nipples, a colostrum sample taken, then the newly-born animal, which was assigned to the mother, was allowed to suckle. In case twins came, one was never allowed to suckle the mother, but was put directly upon cow's milk as a control. Test samples were taken from mother and young at frequent intervals for some time following the birth. In all tests in this series 0.10 c. c. fresh guinea pig serum (per tube) was used as complement.

Exp. IV. Goat No. 29. (Normal; pregnant).

Six subcutaneous injections of sheep erythrocytes were spaced over 16 days in gradually increased doses, a total of 450 c. c. blood cells. On the thirty-fourth day after the last injection the animal gave birth to two kids. Blood samples were taken from both before they were allowed any milk. One was removed and placed upon cow's milk, while the other was allowed to take mother's colostrum after the collodion seals were removed and a sample drawn. The serum from a blood sample taken from the mother before first injection of sheep blood showed a definite amount of normal hæmolysin (0.10 c. c.) against the sheep corpuscles used in the test.

During the course of the immunization the mother's serum showed the highest antibody concentration (0.008 c. c.) nine days after the last

injection; the content rapidly fell so that on day of birth of young (thirty-fourth day after last injection) it was quite low. Since 0.10 c. c. of the mother's serum showed a definite amount of natural hæmolysins against the sheep corpuscles, no greater volume of the test samples was used. The results of the comparative tests are as follows:

Time	Adult's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young.....	0.06 c.cm.	0.018 c.cm.	0	0
1st day after birth.....	.....	0.08 "	0.048 c.cm.	0
2nd " " "	0.070 c.cm.	?	0.045 "	0
4th " " "	.....	0	0.050 "	0
7th " " "	.....	0	0.058 "	0
10th " " "	0.10 c.cm.	0	0.060 "	0
13th " " "	Trace	0	0.060 "	0
17th " " "	"	0	0.075 "	0

It is shown by the above data that the adult rapidly lost its specific hæmolysins—even the natural hæmolysin seemed to have diminished. According to the test, neither of the kids showed any trace of hæmolysin in 0.10 c. c. of their sera taken on the day of birth; the one on cow's milk continued negative, while the one with the mother acquired, by absorption from the colostrum-milk, a definite hæmolytic content in its blood. The colostrum showed a relatively high amount of hæmolysin at time of birth of young, but this rapidly diminished until the test failed to show its presence.

Exp. V. Goat No. 31 (Normal; pregnant).

Received every third day an injection of sheep corpuscles until six were given; total volume injected was 390 c. c. Samples were taken both before blood injections were made, and at intervals during the immunization. Six days after the last injection the animal was found with two normal young. Since the mother's nipples were sealed, neither kid had any milk. Blood samples were taken from each; then one was removed and placed on cow's milk; the other was allowed to suckle mother after the collodion seal was removed and a sample of colostrum was taken. Unfortunately this experiment was of short duration, since the kid with mother became ill (probably from cold) the second day after birth, and experiment had to be discontinued. However, the results are of sufficient interest that they are briefly tabulated here.

Time	Mother's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Colostrum)	Kid "B" Serum (Cow's Milk)
Day of birth of young.....	0.0075 c.cm.	0.015–0.02 c.cm.	0	0
1st day after birth.....	.....	0.035–0.04 "	0.05 c.cm.	0
2nd " " "	0.0097 "	Trace	.....	0

This test shows that, while both blood serum and colostrum from mother contained a high antibody content, neither kid had sufficient antibody in blood at birth to give even a trace of hæmolysin when 0.10 c. c.

serum was used, but the one which had taken mother's colostrum on day of birth showed a fairly high amount of the specific antibodies in its serum on the following day.

Exp. VI. Goat No. 15 (Pregnant).

This animal had been immunized (sheep erythrocytes) five months previously, but at the time this experiment was undertaken no specific hæmolysins were demonstrable in the blood serum. Again subcutaneous injections of sheep blood cells in gradually increasing doses (six) were given, three to four days apart, making a total of 250 c. c. in all. A test showed that the serum reached its highest value (0.001 c. c.) on the seventh day after the last injection, but at the time of birth of kid it had greatly decreased (0.020 to 0.025 c. c.). One normal kid was born 115 days after the last injection. Shortly after birth a blood sample was taken from the kid; the seals were removed from the mother's nipples and a sample of colostrum was drawn. Then the kid was allowed to remain with the mother and suckle. On testing the samples the mother's serum showed a relatively low content (0.020 to 0.025 c. c.) of hæmolysin, which remained quite constant for some days after the birth of young. The colostrum, while scant, was especially high in its hæmolytic content (0.009 c. c.) on day of birth. On the following day the milk showed a very low test (0.15 c. c.) and was entirely negative by the end of the week. A sample of 0.30 c. c. of the kid's serum at birth did not show a trace of hæmolysin by test; so probably little, if any, passed the placenta from the mother. On the second day after having suckled only a trace of hæmolysins could be demonstrated in the kid's serum; this can be explained in part by the known fact that the mother had only a small amount of colostrum, the volume of milk secreted was scant for several days, and the kid showed signs of hunger and under-nourishment.

Exp. VII. Goat No. 6 (Pregnant).

It may be of interest to record in this connection an observation made on another goat which might be classed with this series. The mother showed signs of oncoming labor during the late afternoon, so both blood and colostrum samples were taken. Next morning it was found that the animal had given birth to two large (weight, 4 pounds each), apparently fully developed young, which were dead. Nothing in particular was shown on autopsy, excepting some subcutaneous œdema in each; some bloody fluid was found in the thoracic cavity, which was removed and saved for test; the heart and large vessels were fairly well filled with partially clotted blood, which was removed, and the serum collected; lungs, non-dilated, on section showed nothing unusual; the other organs appeared normal. Possibly a large bleeding (between 300 and 400 c. c.), which was taken from the mother one week before, might have had some influence upon the welfare of the young. The weight of the adult on day following the birth of young was 83 pounds. Upon centrifuging the fluid from the thorax and the blood from heart and great vessels it was found that in one kid the fluids came out quite clear, while those from the other were rather deeply colored by the presence of hæmoglobin. We shall, therefore, consider the tests made upon the clearer fluids from the one kid. The following comparative values were shown: Mother's serum, 0.0025 c. c.; colostrum, 0.0015 to 0.0020 c. c.; kid—the fluids from the blood and from the thorax failed to show a definite hæmolysis in amounts up to 0.20 c. c.; a very slight tint was observed, but it is questionable if this were due to specific antibodies or traces of hæmoglobin in the sample.

This adult was unique in the sense that she seemed to retain a very high antibody content in her blood long after the last blood cell injection. The last blood injection was given about six months (170 days) before birth of the young. Most animals lose their antibodies much more quickly; few show them in appreciable quantities after six months. This animal was immunized before conception had taken place,

since the period of gestation in goats is stated to be five months. If any hæmolytic immune bodies passed through the placenta to the fœtus in this case, the amount was very small.

From the experimental evidence given in this last series of experiments it would appear that the colostrum plays a greater rôle in the transmission of hæmolytic immunity to the offspring than the placenta; the latter probably transmits little, if any, appreciable amount of hæmolytic immune body to the fœtus in utero.

(b) Active Immunization of the Adult Following Immediately or Shortly After Birth of Young.—A number of workers have succeeded in highly immunizing female animals during the period of lactation against toxins and getting the antibody in the milk in a relatively high concentration. Since it has been proved that the milk is an important factor in transmitting antibodies to the suckling during the first days of life through gastro-intestinal absorption, it was thought worth while to undertake similar experiments at later periods in connection with our studies.

Series III. Animals Normal at Time of Parturition.

Exp. VIII. Goat No. 8 (Normal; preliminary test).

This animal gave birth to one normal kid, which was allowed to remain with and suckle mother. Blood samples were taken from the mother and kid; then the mother received a subcutaneous injection of 75 c. c. of sheep corpuscles. Following this, 100 c. c. corpuscles were given daily for three consecutive days, a total of 375 c. c. Blood samples were drawn from the mother and kid on alternate days for two weeks, after which samples were taken every third day for some time longer. In this experiment no milk samples were taken during the first weeks, as the supply was scant; the serum samples were tested, at the same time using 0.20 c. c. rabbit serum per tube for complement. It was found that antibodies did not appear in definite amounts in the mother's serum until five days after the last blood injection; then they rapidly increased until the eleventh day, when the highest concentration (0.0035 c. c.) was found; the antibodies slowly declined until on the forty-sixth day after last injection the value had fallen to 0.037 c. c. Serum samples from the kid when tested in amounts as high as one-half c. c. failed to show positive results. It is questionable if any appreciable amount of hæmolysin was excreted in the milk when the mother's serum was at its greatest concentration. If so, the kid at that age failed to absorb (unchanged) a sufficient quantity to give a definite hæmolytic reaction in the test.

Exps. IX and X.

Similar experiments were performed with goats Nos. 4 and 5, with the exception that milk samples were taken throughout and tested at the same time as the blood serum samples of the respective mother and kid. In neither case did we succeed in getting very high immunity in the mother animals, and it was found that an appreciable amount of antibody failed to pass over in the milk. Naturally the serum of the kids did not show antibodies.

These experiments proved quite unsatisfactory. It appears that an exceedingly high immunization of the female is necessary before a fairly high antibody content occurs in the milk. In neither of the two goats last mentioned was the degree of immunization sufficient to cause a demonstrable overflow of antibodies in their milk.

An obvious objection to the active immunization of normal females immediately after birth of young is the necessary delay before the production of antibodies in high concentration in the mother's body, and their appearance in the milk. In the meanwhile the young animal becomes more mature; its digestive powers are more pronounced and probably anatomical changes occur in the gastro-intestinal mucosa which retard or prevent the absorption of the unchanged antibodies.

Series IV. Animals Partly Immunized at Time of Parturition.

In order partly to overcome certain objections to which attention has just been directed, animals which had been previously immunized and still showed a low antibody content in their blood at the time of birth of the young were reimmunized by more blood injections following parturition. In this way it was hoped that a high immunity could be induced in the mother, and more quickly than was possible in the strictly normal animal, and that the milk would either remain high or increase in immune body value. Possibly kids receiving such milk in quantity during the first weeks of life would be able to absorb the immune body unchanged and as a result their sera would remain about constant, or even show some decided rise in the hæmolysins. In opposition, other factors enter, such as, (a) animal growth (increase in body fluid), (b) loss through blood samples withdrawn, and (c) the metabolic destruction of antibodies. Therefore such experiments should be well controlled.

Exp. XI, Goat No. 6 (Previously immunized).

Found in the forenoon with normal twins, and, as they had suckled, only a small sample of milk could be obtained from the mother. Both remained with mother until late in the afternoon. The mother was given a large injection of washed sheep corpuscles the same evening.*

The blood injections were repeated every second day until four in all were given, a total of 425 c. c. Blood samples (adult and kid) and milk (adult) were taken for several weeks. All were tested simultaneously. The results of the test may be tabulated as follows:

Time	Mother's Serum	Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young.....	0.0080 c.cm.	0.05–0.06 c.cm.	0.002 c.cm.	0.0035 c.cm.
2nd day after birth.....	0.0077 "	0.27 c.cm.	0.002 "	0.0030 "
4th " " "	0.0040 "	0.16 "	0.003 "	0.0035 "
6th " " "	0.0020 "	0.08 "	0.003 "	0.0040 "
10th " " "	**0.0009** "	0.05 "	0.004 "	0.005 "
12th " " "	0.00098 "	**0.045** "	0.0042 "	0.005 "
16th " " "	0.0012 "	0.05 "	0.006 "	0.007 "
22nd " " "	0.0017 "	0.15 "	0.0078 "	.....
28th " " "	0.0022 "	0.15+ "	0.009 "	0.019 "
34th " " "	0.0027 "	0.18 "	0.015 "	0.03 "
40th " " "	0.003 "	0.27 "	0.022 "	0.045 "

(When 45 days old, Kid "A" weighed 14½ lbs. and Kid "B" 17 lbs.).

As evident from the above data, the kid which was with the mother and getting a bountiful supply of milk containing large amounts of immune body showed in its blood serum an antibody content practically running parallel with that of a control (brother) which was fed upon cow's milk after the first day following birth. The ratio of weight between the two kids probably ran roughly parallel throughout the test. So it would appear at least in this instance that not many of the antibodies pass over unchanged to the circulation from the gastro-intestinal tract of a suckling animal after the first days following birth.

This experiment would suggest that, even when we can get an animal highly immunized and the milk shows the presence of considerable antibodies, hardly an appreciable amount is absorbed unchanged from the

*At the same time this animal received injections of killed cultures of B. coli in small but increasing doses on days alternating with the blood injections, these injections were continued over two weeks without any harmful effects in so far as noted.

digestive tract by the suckling after the first days of life. These results were supported by other observations, but in experiments where a control twin was not available.

(c) Active immunization of newly born or older kids against foreign blood cells.

Series V. Blood Injections into Kids.

In connection with our studies where mothers received very large injections of blood during gestation, especially in the very last weeks, we must consider the possibility of soluble antigens passing the placenta to the fœtus. Bang and Forssman[68] state that ox corpuscles gave an ether soluble fraction which possessed antigenic properties when injected into suitable animals. It is possible, therefore, that in the body also similar soluble products are liberated in quantity and eventually reach the blood stream from the large subcutaneous blood injections. Hektoen and Carlson[69] have shown by transfusion experiments with dogs which had been previously injected (intravenously) with small amounts of goat's blood cells that after several hours the antigen is fixed in the body of the injected animal, and not transmitted in sufficient quantities to the normal recipient to cause specific antibody production in the latter. But when large blood injections were given to a donor, then later a direct transfusion was made to a normal animal, the recipient, as well as donor, produced antibodies in due course of time. This showed that the body's ability to fix the formed antigen was limited. Therefore, the possibility exists that from large subcutaneous blood injections into gravid goats soluble antigen might eventually reach the fœtus. The question arises, If the fœtus receives antigen shortly before birth, has it the power to react and form antibodies, or may it retain the antigen and form antibodies shortly after birth? Any attempt directly to inject the fœtus * in utero is necessarily surrounded by numerous technical difficulties; moreover, the results thus derived would be justly subject to criticism. A few experiments were carried out on kids at different periods after their birth to test their reactive ability against sheep erythrocytes. The results may throw some light upon this side of the immunity problem.

Exp. XII. Kid No. 96B (Newly-born).

This kid was a twin born from a mother which had been immunized against sheep corpuscles over five months before parturition. The mother's blood taken six days before birth of the young showed a trace of hæmolytic immune body in 0.10 c. c. serum, while a sample taken on the day of birth failed to show any hæmolysin in like amount of serum. The colostrum on day of birth of young showed a small amount of immune body in 0.10 c. c., but the milk on the following day was negative. A blood sample was taken from the kid before it was allowed to suckle; then it was placed with the mother. The same evening 12½ c. c. of sheep corpuscles were injected into the kid subcutaneously and the next afternoon another injection of the same amount of corpuscles was given. Blood samples (8) were taken at intervals during the 15 days following the last injection. On testing** it was found that 0.10 c. c. serum from blood samples taken second and third days after birth showed a small amount of hæmolysin, but this practically disappeared and soon showed only as a trace. Since the kid's blood sample taken before suckling showed no hæmolytic action in the same amount, one must look to the colostrum as a source of its antibody on second and third days. The tests of samples covering two weeks following last blood injection failed to show more than a trace of hæmolysis at any time, but perhaps this trace was slightly greater at the end of the second week. Adults usually show specific hæmolysin production by the end of the first week after injection, and often this is at a maximum on the ninth to twelfth day.

*Kreidl and Mandl (loc. cit.) attempted injection of the fœtus (goats) in utero, but were unsuccessful in the outcome in practically every case.

**Used the regular technic excepting 2.0 ccm. of a 5% instead of a 7½% suspension of sheep erythrocytes were added per tube.

We must conclude that the young in this case produced hæmolysins only to a very slight extent (minute quantity) in response to the injection of sheep blood corpuscles in relatively large amounts.

Exp. XIII, Kid No. 15B (10 days old).

This kid was also born from an immunized mother, but failed to show any appreciable amount of specific hæmolysins in its blood serum at the end of the first week after birth. On the tenth day after its birth the kid was given a subcutaneous injection of 10 c. c. of sheep blood corpuscles and an equal dose was given in the same way on the twelfth day. Samples were collected at intervals for three weeks after the last injection. By the test* all were negative (0.10 c. c.) until the ninth day, when a slight trace of hæmolysin was shown; on the twelfth day this was somewhat increased, a definite but slight hæmolysis; sixteenth day, questionable trace; twentieth day, 0.10 c. c. gave negative results. This would indicate that a slight but transient immunity reaction took place against the sheep blood cells.

Exp. XIV. Kid No. 68B (18 days old).

The mother of this kid had been immunized against sheep blood cells some time previously. It was removed from the mother on day of birth and fed during the first days upon goat's milk and then later upon cow's milk. On the eighteenth day after its birth 20 c. c. sheep blood cells were injected into it subcutaneously and two days later an equal volume was given in the same way. Blood samples were taken during three weeks following the last injection. Testing by the regular technic, it was found that 0.10 c. c. of serum showed a trace of antibody on the eighteenth day after birth (first injection of blood). Probably it got colostrum, which contained immune body, on day of birth. These antibodies quickly disappeared and further samples were negative in amount tested until six days after last blood injection, when a slight trace again appeared and gradually increased, so that on the twelfth day after the last injection of blood a slight but definite hæmolysis was produced by 0.10 c. c. of serum; this had again practically disappeared within a week. This experiment indicates that only a very slight immunity reaction occurs in a kid after it has reached the age of eighteen to twenty days.

If we are permitted to draw any conclusions from these few experiments we should say that the newly-born animal has only slight ability to form hæmolysins during the first days following birth. Consequently we cannot assume that the fœtus in utero has greater power, even if antigen should reach it through the placenta. But, on the other hand, it is more probable that the fœtus has even lesser power to form immunity reaction products than the newly born. This seems to be a property which is only gradually acquired by the growing young, as they adjust themselves to their environment in the external world. The work of several investigators (Schkarin,[70] Moll[71]) would seem to substantiate the view that the organism of very young infants or animals has only a slight ability for antibody production.

(d) Gastro-intestinal absorption of antibodies.

Series VI. Feeding of Hæmolytic Serum to Newly-born Kids.

As apparent from the data submitted, the newly-born kid readily absorbs in an unchanged condition the specific hæmolytic immune bodies from colostrum through the gastro-intestinal tract. These immune bodies do not appear in the kid's blood serum unless present in considerable amounts in the ingested colostrum. However, the amount in colostrum-milk may be comparatively low, and still cause a decided increase in hæmolytic value of the suckling's blood, if the total volume taken is large. The question suggested itself, Was this free absorption of immune body taken through the milk-colostrum peculiar to the combination, or, are

*Used the regular technic excepting 2.0 ccm. of a 5% instead of a 7½% suspension of sheep erythrocytes were added per tube.

the immune bodies readily absorbed by the newly-born kid, when fed hæmolytic serum, irrespective of the nature of the medium in which they are present?

As we had an opportunity, this question was put to test in two cases by feeding newly-born kids with homologous serum (goat) which contained specific hæmolysins. The kids were taken away from the mothers immediately after birth and were never allowed to suckle. Owing to the lack of experimental animals at the time this investigation was in progress, exact quantitative experiments upon this question, unfortunately, could not be continued. The following results may prove to be of some interest in that direction.

Exp. XV. Kid No. 112B (Newly born, weight 1,350 grams).

A twin, which was not allowed to suckle mother, was removed immediately after birth and a blood sample was drawn. An immunized goat (No. 15) was bled at once, the blood defibrinated and centrifuged to remove all corpuscles. About 30 c. c. of this supernatant serum was fed to the kid and no other feeding was made until the next day when it was given cow's milk, which was continued from that time on. Unfortunately, on testing this serum it was found to be of much lower value than suspected; test showed that 0.02 c. c. produced, however, a well-marked hæmolysis when tested by the regular technic, with the exception that 2.0 c. c. of 5 per cent. instead of 7½ per cent. sheep blood suspension was used.

Samples of blood were taken upon four consecutive days after the feeding of the immune serum. When these samples (including the sample taken from the kid before feeding) were tested in the manner just mentioned none showed hæmolysis in amounts of 0.15 to 0.20 c. c. If one-third of the antibodies were absorbed (unchanged) one would expect the test to give at least a slightly positive reaction in the volume of kid's serum which was tested. It follows that the absorption of unchanged antibodies from the digestive tract in this animal was not pronounced.

Exp. XVI. Kid No. 97B (Newly born, weight 2,535 grams).

Immediately after birth this kid was removed without being permitted to suckle. A blood sample was taken, then it was fed by pipette 15 c. c. of specific immune serum (Goat No. 15) which had been kept under good condition in freezing ice-box for almost four months. This hæmolytic serum, tested shortly after the feeding experiment, was of such potency that 0.002 c. c. almost completely hæmolysed 3 c. c. of 5 per cent. sheep blood suspension (0.10 c. c. guinea pig serum for complement), while 0.001 c. c. gave a definite hæmolysis. About one hour after this feeding the kid was given 150 c. c. of cow's milk. No further feeding was made until the next morning, when it was put upon cow's milk regularly. Blood samples were drawn daily at first, then at longer intervals, covering in all fifteen days' observation. The tests were carried out upon these samples by the same technic (amount of blood samples, complement, and volume fluid) employed in the preceding experiment (Kid No. 112B). It was found that the sample taken before the feeding of hæmolytic serum was negative in 0.10 c. c.; the day following the immune serum feeding 0.10 c. c. serum showed a well-marked action; the second day after the same amount of serum showed less hæmolytic action, the hæmolytic power gradually diminishing until on the thirteenth and fifteenth days after the serum feeding no action was shown by 0.10 c. c. This experiment showed that considerable absorption (approximately 10 per cent.) of the unchanged hæmolytic serum from the digestive tract took place and appeared in the general circulation of the young animal. Recognizing the fact that no definite quantitative conclusions can be drawn from as few as two experiments, no more can be said than that these experiments suggest that antibody absorption, while taking place, is relatively meager when hæmolytic sera are fed to newly-born kids.

5. GENERAL DISCUSSION OF RESULTS

Before attempting to draw any definite conclusions from the above experimental data, it may be well to consider more fully some of the findings and the related questions. In this brief discussion not only our own results, but the correlated results of other workers, will be considered. The experiments clearly demonstrate that goats, when highly immunized against sheep erythrocytes during the latter part of gestation, transmit the specific antibodies to their sucklings. Apparently the placenta plays a very small part in this transmission, but the principal rôle is played by the antibodies which are present in the colostrum-milk and are readily absorbed from the digestive tract when taken by the newly-born offspring. In those cases where the young born from immune mothers were prevented from taking the colostrum-milk until after a blood sample had been drawn tests failed to show the presence of hæmolysins in the kid's blood serum in sufficient amount to be appreciable by the method used. So we must conclude that the fœtus in utero acquires from the actively immunized mother little, if any, hæmolytic immune body. We must therefore direct our attention particularly to the antibody-bearing colostrum-milk and the gastro-intestinal absorption of antibodies by the young.

Observations upon the colostrum taken at time of parturition and before the young were permitted to suckle proved to be very interesting. When compared with the same animal's blood serum taken at the same time and tested in parallel it was sometimes found to be considerably higher in immune body value; in some instances reaching a value two to three times greater than the blood serum. The published data of other workers would indicate that this occurs not infrequently where animals are immunized some time before birth of young against various antigens (Stäubli,[32 80] Wegelius[24]), but particular attention has not been given to its importance. The question arises, Is the high hæmolytic value of the colostrum in these cases due to (1) accumulated specific antibodies formed primarily in the mammary gland, or (2) a storage of antibody removed from the blood stream, or (3) are these antibodies present only in a concentration equivalent to that of the other body fluids, but are augmented in action by other substances present in the colostrum, *e. g.*, substances analogous to the "bovine colloid" of Bordet and Gay[72]?

As yet we know little concerning which tissues or organs form the hæmolysins. The theory has been advanced, with some experimental support, that the blood-forming organs are the principal factors in the production of antibodies (Hektoen[73]). According to Hektoen,[69 74] the blood itself, and probably the subcutaneous tissues at site of injection of blood corpuscles do not fix the antigen and produce hæmolysins to any appreciable extent. If this view concerning the formation of antibodies is accepted, little reason exists to suppose that the hæmolysins are being formed to any extent in the mammary gland. No experimental proof has been brought forward showing that the cells of secreting glands produce antibodies. So, on the whole, little evidence exists which would support the first possibility.

Probably the second possibility mentioned is the true explanation of what actually occurs. In the absence of any direct experimental work upon this particular question certain facts may be considered, and a hypothesis deducted which will support the view. The quantitative relationship between the protein constituents of normal colostrum and normal milk, in conjunction with known facts, must be considered. In the absence of full chemical data upon goat's colostrum and milk, the data upon analogous products from the cow must be used. According to König's[75] tables, the composition of goat's and cow's milk is very similar. Hoppe-Seyler (Thierfelder[76]) states that colostrum is probably somewhat poorer in casein than milk, but very much richer in albumin and globulin.

König's[75] tables show practically the same general results with the exception of the casein, which he gives in a higher percentage for the

average colostrum (4 per cent.) than in the milk (3 per cent.); he states as the average for coagulable protein (albumin and globulin) in colostrum 13.6 per cent., for milk (albumin) 0.53 per cent. The colostrum contains an especially high content of globulin (Eichelberg[77]), while the milk contains only traces of this protein. At once we are struck by the great extremes of protein content existing between the colostrum and milk from the cow. But it is also noticeable that cow's colostrum and milk do not show this great quantitative variation between their casein contents. Some observers report that the average casein content is about the same in colostrum and later milk. Casein is considered to be a nucleo-albumin, which is elaborated by the glandular epithelium, and according to Mandel[78] probably originates in the gland cells from a breaking down of the nucleo-protein. So the casein content of the colostrum or milk may be used as an index of the secretory activity of the gland. The lacto-albumin which is present in the milk is also probably elaborated in the gland cells, and with the casein constitutes the normal milk proteins. The trace of lacto-globulin present in later milk has the properties of the sero-globulin, and probably is simply excreted by the gland cells by removal from the blood stream in an unchanged condition. In support of this supposition the work of Bauer[79] may be quoted. He prepared anti-sera by the injections of serum, colostrum and milk. By a refined complement fixation test he was able clearly to differentiate blood serum and milk from the same animal. But when the anti-serum from cow colostrum was used he found on testing that the highest specificity was shown against the same colostrum (1-100,000), but also he had a fixation with cow's milk (1-10,000) and of ox serum (1-10,000). These results showed that in colostrum there is present, not only the special milk proteins, but also those peculiar to the same animal's blood serum. His experiments would indicate also that the milk proteins were a specialized product of the milk glands. From both the purely chemical and the biological reaction standpoint it would appear that the proteins of colostrum and milk are somewhat different in qualitative character and in origin. The colostrum contains, in addition to those proteins of the normal milk, blood serum proteins, especially globulin, in considerable quantity.

It is a well-known fact that during the active immunization of an animal its blood serum shows a decided increase in its protein content.* Also it is equally well known that antibodies themselves, if not protein in character, are closely associated with the proteins, in particular the serum globulins. Since we have seen that globulins are present in considerable quantity in colostrum and that the biological test showed that colostrum contained serum protein, while milk did not, we can assume that this is a sero-globulin, as only a trace of globulin is found in normal milk. The high concentration of antibodies in the colostrum might be explained by the excretion of serum proteins (globulin, etc.) by the glandular epithelium, and incidentally the specific antibodies which are probably closely associated. The antibody value, if the hypothesis is true, would parallel the protein concentration in the colostrum.

Stäubli[32] [80] made similar observations upon typhoid agglutinin-colostrum and blood serum. He found that the colostrum from a clinical case, as well as animal experiments, showed much higher agglutinin content than the blood serum at the same time. He thought that perhaps the milk-glands took up the antibody from the blood and returned the water, thus causing a concentration, and, also, that a certain parallelism ex-

*Ledingham (Jour. of Hyg. 1907, VII. 65) reported quantitative protein changes in serum of a goat during an immunization against diphtheria toxin. The total protein in the normal serum before injection of toxin was 5.867%; the maximum reached during the course of immunization was 8.555%, then gradually diminished.

*Gibson and Banzhaf (Jour. Exper. Med., 1910, XII, 411) never found the total proteins to rise above 10.52% during the active immunization of horses (11 animals) against diphtheria or tetanus toxins.

isted between the coagulable proteins (lacto-albumin, lacto-globulin) and the agglutinins. He considered the gland cells as playing an important *active* rôle in the formation of agglutinins, and that the high value of colostrum was influenced by the destruction of those cells.

The possibility that substances may be present in colostrum which might augment the action of the hæmolysins present offers some points of theoretical interest. But the supposition is hardly tenable as an explanation, since the dilution of the colostrum was so great in many instances that a "colloidal action" would be negligible. Other chemical substances present would also suffer in the same way. These substances would also appear in the normal colostrum drawn from the animal before immunization, but in no case did we ever get a hæmolysis from such a colostrum in the presence of rabbits or guinea pig complement, even when used in large quantities.

Before leaving this side of the question we wish to say that it is highly desirable that further work should be carried out along these particular lines.

The gastro-intestinal absorption of unchanged antibodies from immune serum has received attention ever since the introduction of serum therapy. Escherich [81] attempted to passively immunize children by giving antidiphtheritic serum per os. He reported that infants of the first months showed a slight rise of antitoxin in their blood, but in older children he met with no success. Laboratory animals failed to absorb appreciable amounts of antitoxin, even when fed in relatively large quantities (Carriere [82]). The work of the von Behring school (Behring,[57] Ransom,[17] Roemer [88]) confirmed the findings that adult animals under normal conditions do not absorb antitoxic serum (heterologous) in an unchanged condition, but the newly-born animals do so readily from the mother's milk (homologous). They attempted to explain this on the basis of the differences between the anatomical structure of the gastro-intestinal mucosa in the newly-born and the grown (Disse [84]), but these claims have not been verified. At the present time certain other factors are considered very important in this process. Salge [85] fed antidiphtheritic serum to infants, but states that no antitoxin passed over to the blood. He observed that infants getting antitoxin through the milk of the nurse readily absorbed it. He considered this difference to be due to homologous antibodies in the milk which were easily absorbed, while the heterologous antidiphtheritic serum was not assimilated in an unaltered state. Salge,[86] in order to test this point, fed infants for several weeks with goat milk (heterologous) drawn from immunized animals and showing considerable antitoxic value, but was unable to demonstrate the presence of antibodies in the blood of the infants. The work of Bertarelli [87] and others would tend to support the same view, although this point is not fully settled.

In my experiments it was seen that newly-born kids readily absorbed hæmolysins when taking the mother's colostrum-milk containing these bodies. Also in one case in which the homologous hæmolytic serum (highly potent) was fed it was absorbed in considerable amounts, but probably not so readily as the colostrum-milk hæmolysins. Uffenheimer [88] in his extensive work upon gastro-intestinal absorption (bacteria, proteins, antibodies) touched upon the absorption of hæmolysins. He fed guinea pigs, newly born or during the first days of life, considerable amounts of hæmolytic serum derived from rabbits immunized against guinea pig blood cells. He concluded that none of the hæmolysins was absorbed, since the young animals gave no evidence of intoxication (hæmoglobulinuria, decrease in number of blood cells, etc.). Much smaller doses of the same serum when injected into controls caused their death.

The reports of several investigators (Ganghofner u. Langer,[89] Uffenheimer,[88] Roemer u. Much [90]) upon the question of the gastro-intestinal absorption of unchanged proteins, etc., by young animals, call attention to various factors which influence the absorption, such as age, overfeeding of proteins, the nature and source of the proteins, digestive powers,

irritation or lesions of the intestinal mucosa, species of animal, individual variation, etc. In considering the possibility of immunizing the young animal through the digestive tract, the above factors must be kept in mind.

The failure to transmit immune bodies to the suckling when the mother is immunized after its birth, in all probability, is chiefly due to two reasons; first, the milk immune bodies do not appear in the milk at any time in very great quantities, while, secondly, the time of their appearance is past the critical age point where the young are able to absorb the hæmolysin (unchanged) to any extent.

When immunization against sheep corpuscles was carried out in goats during the period of lactation the milk antibody content, when compared with that of the serum, was never greater than 1:45-50. Similar observations upon goats immunized against diphtheria and tetanus showed that the milk contained relatively greater amounts of antitoxin when compared with the serum than shown in the above ratio for hæmolysins (Ehrlich u. Wassermann[91]).

All observers are agreed in the conclusion that the newly-born animal (or infant) absorbs from the digestive tract unaltered antibodies most readily when given immediately after birth. The most effective means of supplying the antibodies to the young is through the mother's colostrum milk (homologous); probably next in importance is the homologous serum, and finally the antibody-milk or serum from some other species.

It follows from our observations that of all factors the colostrum plays the most important rôle in transmitting antibodies from the actively immunized mother to the young. Even under normal conditions, it is reasonable to suppose that protective substances are concentrated in the colostrum from the mother's body fluids, and may be in part absorbed by the infant after the first feedings. Consequently, it seems of highest importance that the newly-born child should, during the first days of life, receive the colostrum-milk, unless it is contraindicated for some special reason. Perhaps the natural resistance which many infants show against many infectious diseases may be partially explained on the basis that the child is passively immunized by anti-substances excreted through the immune mother's colostrum and milk which are absorbed by nurslings during the early days of life. Such a view gains strength from the observations of Moro,[92] who found that the blood serum from breast-fed infants showed greater bactericidal and hæmolytic powers than that from infants who were artificially fed upon cow's milk.

The practical application of adding to the mother's immunity by vaccines during pregnancy would undoubtedly be attended with risk of disturbing the natural course of pregnancy, and should not be entered into unless further investigation demonstrates its safety.

6. To summarize briefly the principal results of the foregoing experiments, it was found that:

1. Goats actively immunized against sheep blood corpuscles during gestation passively transmitted the specific hæmolysin to their young.

2. The colostrum was the chief agent in bringing about the passive immunization of the suckling. Sucklings which got the colostrum and first milk rapidly acquired a relatively high antibody content in their blood, which was well retained.

3. When the immunization was done during the period of gestation the colostrum contained a high content of specific hæmolysin, often much higher than the adult's serum at time of parturition.

4. The hæmolytic antibodies rapidly disappeared from the milk after the mother had been suckled by the young.

5. The blood taken from the newly born before they were permitted the antibody colostrum showed no appreciable amount of hæmolysin by the test used. The placenta played a minor rôle in the passage of hæmolysins to young before birth, practically negligible in most cases.

6. Mother goats actively immunized against sheep blood corpuscles immediately after birth of their young failed to transmit any demonstrable immunity to their suckling young.

7. The milk, in some cases, contained no demonstrable hæmolysins, but in others showed fairly large amounts.

8. Apparently a very high degree of immunity is necessary before appreciable amounts of antibodies are excreted through the milk.

9. Older sucklings apparently did not absorb the antibodies in an unchanged condition.

10. The young animals (kids) did not respond to any extent in production of hæmolysins following subcutaneous injections of foreign blood cells (sheep).

In conclusion, I wish to express my indebtedness to Dr. William H. Park, Director of Laboratories, Department of Health, for many helpful suggestions in connection with the above work. I am also indebted to Drs. Paul Bartholow and Maria Grund for valuable assistance.

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ON THE RELATIVE VALUES OF CERTAIN NORMAL SERA USED AS HÆMOLYTIC COMPLEMENT

(Studies on Serum Hæmolysins, III)

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In a report upon "Experimental Data Relating to Hæmolytic Sera," published in the Collected Studies for 1908-09, attention was directed to an experiment where guinea pig serum was more active than rabbit serum when used as complement for like hæmolytic immune bodies from rabbit and goat, although the relationship was not parallel. In the experiment cited a constant volume (0.05 c. c.) of immune serum was added to each of four series of test tubes; two series contained immune serum from rabbit, the other two immune serum from goat. Each of the series contained fifteen test tubes, to which fresh serum for complement was added in decreasing amounts between the limits of 0.50 c. c. to 0.01 c. c. Guinea pig complement was added to one of the series containing rabbit immune body and also to another containing goat immune body. In like manner rabbit complement was added to parallel series containing immune body from the same sources. The immune body from each animal was specific against sheep corpuscles. It was found in this particular case that the guinea pig complement gave a decidedly greater degree of hæmolysis with the rabbit immune body than with the goat immune body. On the other hand, the rabbit complement gave reverse results, although not to the same extent—it showed somewhat greater activity* (hæmolysis) with the goat immune body than with the rabbit immune body.

TABLE I

Comparative Values of Different Immune Bodies (against Sheep Corpuscles) with Guinea-Pig and Rabbit Complements

Complementing Serum { Source / Amount	Guinea-Pig 0.10 ccm.			Rabbit 0.20 ccm.		
Source of Immune Body	Guinea-Pig	Rabbit	Goat	Guinea-Pig	Rabbit	Goat
Amount of Immune Body necessary to produce a given degree of hæmolysis	0.0025 ccm.	0.0003 ccm.	0.0125 ccm.	0.0048 ccm.	0.00125 ccm.	0.0038 ccm.

In view of these interesting results, it was decided to repeat the experiment upon somewhat broader grounds with immune sera derived from different species of animals immunized against the blood corpuscles of sheep. The object was to see if these results could be confirmed, and also to furnish practical data for the selection of a complementing serum for the immune body from a particular animal species. Under certain conditions where great series of hæmolytic tests are carried out it might be advantageous to use rabbit serum for the complement rather than the

* In the above work, as well as the work which follows, complement efficiency was considered from the standpoint of toxic effect (hæmolysis) in distinction from its combining action. Muir's work has fully demonstrated those properties of complementing sera.

guinea pig serum, a question of laboratory economy. For the purpose of the new experiments two guinea pigs, a rabbit and a goat were immunized by repeated injections of well-washed sheep's blood cells in gradually increased doses. All were bled on the same day into chemically cleaned test tubes, which were placed in an ice-box, where the serum was allowed to separate from the clot. The serum was removed from the clot in each case, placed in paraffine-coated, chemically cleaned bottles, then stored in ice-box, where they were kept several weeks at a temperature only slightly above the freezing point. Each serum was inactivated by heating at 56° C. for 30 minutes shortly before the tests were performed.

The technic was carried out by adding to each tube, first, the given amount of immune body, then salt solution to balance required volume, followed by given amount of complement; finally the blood suspension (1.0 c. c. of 10 per cent. by volume). The total amount of fluid was 3.0 c. c. The components were thoroughly mixed at once on the addition of the blood and again before the tubes were placed in the incubator. Incubation was done between 36° C. and 37° C. for one hour; again the tubes were well shaken, then placed in ice-box until the intact corpuscles were completely sedimented. Results were taken by making comparative equi-colorimetric readings in each test. These are indicated in the tables. Preliminary tests were made in each case in order to get approximate valuations of the different immune bodies with given amounts of different complementing sera. The results given in Table 1 were obtained by using only guinea pig and rabbit serum for complement. Since the test cited showed that fresh guinea pig serum was more active than rabbit serum as complement, double the volume of the latter was used in this particular test.

The relative values of the hæmolytic immune body content for the sera from guinea pig (mixed sera from two animals), rabbit and goat, were determined against a constant amount of guinea pig complement (0.10 c. c.) and a constant amount of rabbit complement (0.20 c. c.). Also normal goat and sheep sera were used in the same test, but with negative results with the maximum amount of immune body titrated; therefore, this portion is purposely omitted from the table. As evident from Table I, the results given by each of the complementing sera are roughly parallel. That is, if the reciprocal values of the numbers which indicate the relative strengths of the immune bodies used in the two series are taken and the results graphically represented, the form of both curves will be somewhat similar. But a comparison of the ratios of the immune body equivalents in the two series (complements) shows very irregular results. In this case the rabbit complement appeared to be relatively more active than the guinea pig complement when used with the immune body from the goat. But, as will be pointed out later, this relationship is not constant (see Table II). The table shows that guinea pig complement was more efficient than that from the rabbit with the immune bodies from both rabbit and guinea pigs. Certain workers would hold the complement entirely responsible for the peculiar variations of this character. While admitting the influence of complement constitution as a factor, we are inclined to think that there are other factors which must be considered. But we shall pass on to the next experiment, after which the results will be more fully discussed.

The preceding experiment gave the relative values of the different immune sera when constant amounts of either guinea pig or rabbit complement were used. Therefore, we have reversed the above conditions and prepared series where constant amounts of immune body have been used with variable amounts of complementing sera. However, in this case the amount of immune body was increased about double that given in Table I for series Ia and IIa, while in series Ib and IIb the increase was brought extremely high (0.10 c. c. of each immune body). As first indicated by the work of von Dungern (Münch. med Wochenschr., 1900, XX, 677), and others since, an increase in amounts of immune body used permits a decrease in quantity of complement for a given degree

TABLE II

COMPARATIVE COMPLEMENTING VALUES OF DIFFERENT SERA UPON GUINEA-PIG, RABBIT AND GOAT IMMUNE BODY (AGAINST SHEET CORPUSCLES)

Series	Ia			IIa			Ib			IIb		
Source of Complement	Normal Guinea-Pig Serum			Normal Rabbit Serum			Normal Goat Serum			Normal Sheep Serum		
Immune body — Source	Guinea-Pig	Rabbit	Goat	Guinea-Pig	Rabbit	Goat	Guinea-Pig	Rabbit	Goat	Guinea-Pig	Rabbit	Goat
Immune body — Amount	0.005 ccm.	0.0005 ccm.	0.02 ccm.	0.005 ccm.	0.0005 ccm.	0.02 ccm.	0.10 ccm.	0.10 ccm.	0.10 ccm.	0.10 ccm.	0.10 ccm.	0.10 ccm.
Equivalent Amounts of Complement (equal hæmolysis) ..	0.025 ccm.	0.023 ccm.	0.018 ccm.	0.28 ccm.	0.35 ccm.	0.125 ccm.	1.0 ccm.	0.60 ccm.	0.45 ccm.	∞	∞	∞

of hæmolysis. By following such a procedure theoretically large amounts of immune body should show the presence of small amounts of complement in a normal serum when employed in the regular hæmolytic test. Preliminary tests showed that normal goat and sheep serum contained little or no complement for the particular immune bodies used in our experiment. Therefore, the application of the above principle to this test for the determination of the complementing values of goat and sheep sera in the series. The test was so designed that series Ia and IIa formed one group, while a second group included Ib and IIb. Such an arrangement permitted the two groups to be more easily considered separately. The test was carried out in the regular way. Table II shows quantities of complement and immune body used in each case and the comparative results.

From the limited data which are herewith submitted we do not feel warranted in drawing conclusions, but, taking these results in conjunction with those published by other workers, certain deductions are perhaps justifiable. In the first place, a direct comparison will be made between our last results (Table II) and those found in the experiment referred to in the beginning of this article—the influence of guinea pig complement and rabbit complement upon like immune bodies from rabbit and goat. Table II shows that the guinea pig complement is much more active than that from the rabbit for both immune bodies, which agrees with the experiment cited.* However, a rough parallelism exists between the two complements as regards their activity in conjunction with the two immune bodies—not the reverse as in the early experiment. Therefore, a parallel experiment carried out along the same lines fails to confirm the earlier results. While we have no direct experimental proof to support the supposition, still it is conceivable that these results may be explained by differences in structures of the immune bodies, or variation in the constituents of the complementing sera from different individuals (same species). Certain elements may act independently, or several may act in conjunction with each other. Such factors may in part account for the lack of uniform agreement between the immune body doses derived with the two complementing sera as shown in Table I.

These tests are of special interest from several standpoints, some of which we shall consider in this connection. Perhaps one of the most interesting of these is the question of relationship between complementing and hæmolytic immune serum derived from the same species. Ehrlich (Festschrift zum sechzigsten Geburtstage von Robert Koch, 1903, 509) expressed the opinion, with some experimental support, that normal serum from the same species of animal which furnished the immune body was the most efficient as a complement. The observations of certain other investigators are not in full accord with Ehrlich's statement; several exceptions have been discovered. Our own experiments, as tabulated above (Table II), showed that when used as complement with a homologous immune body only one normal serum out of three** was more efficient than a heterologous complementing serum. This complement—normal guinea pig serum—was not only more efficient with its homologous immune body, but also with the heterologous immune body from rabbit and goat.*** Rabbit complementing serum was more active with immune body from goat and guinea pig than with its homologous immune body; goat complementing serum was more active with its homologous immune body than with guinea pig or rabbit immune body. After our experimental work was finished a paper by Noguchi (Jour. of Exp. Med., 1911, XIII, 78) appeared, which in part reported quite an extensive study along similar lines. He found that, "With the amboceptors produced in guinea pig, cat and chicken the strongest action was obtained with their homologous complements. On the other hand, the amboceptors from dog and

* The results of these tests do not agree with those shown in Table I of this article. Probably due to quantitative differences in complementing serum from different individuals.

** The normal sheep serum was not included among the complements in this connection, since it failed to act upon any of the immune bodies used.

*** Table I indicates that these results for heterologous sera are not absolutely constant.

rabbit acted more strongly with certain heterologous complements than with homologous complements." His immune body in each case was specific against human blood corpuscles. Our results are in complete agreement with those published by Noguchi in so far as the two pieces of work parallel each other. It follows that a complementing serum to be of greatest efficiency need not be from the same species of animal which furnished the immune serum.

Another point of interest in connection with serum hæmolysis is the question of the efficiency of a normal serum as complement when used against blood cells from the same species. Past experiences have shown that, in general, under such conditions little, if any, hæmolysis is produced. Perhaps Bordet (Annal. d. l'Institut Pasteur, 1899, XIII, 273) was the first investigator to report negative results. He observed that when hens' corpuscles were sensitized with specific immune body from rabbit, then treated with normal hen serum, the cells did not dissolve, although in an earlier paper (Annal. d. l'Institut Pasteur, 1898, XII, 688) he cites an experiment where rabbit blood cells were dissolved by fresh rabbit serum after being sensitized by a heated specific immune body from guinea pig. Muir (Studies on Immunity, Oxford Medical Publications, 1909, 71) made careful studies along similar lines. His studies distinguished between the toxic and the combining properties of complements. He concluded from the tests which he reported "that in the cases studied the highest dosage of the complement of an animal occurs when used against its own corpuscles." It was found that in most cases the complement was bound to the immune body-cell complex, and a certain degree of hæmolysis was produced when large doses of both immune body and complement were used. The complement when combined has only a very slight toxic action upon homologous cells, and when it fails to combine, naturally, no hæmolysis is produced.

Since our immune body was prepared by injecting guinea pigs, a rabbit and a goat with sheep corpuscles, an opportunity was offered by which the normal homologous serum could be tested as to complementing (toxic) power. As indicated above (Table II, Series IIb), the results were practically negative. Repeated tests were made with fresh serum from different sheep, in quantities as high as 1.0 c. c., without definite results; at the most only a slight tint was produced, more often no color change. The complementing action of goat's serum was also very deficient (Table II, Series Ib) when used against sheep corpuscles sensitized with appropriate immune body. The tests were repeated with serum from different goats and with the same results. Those results may be explained by the close family relationship between goat and sheep, and an inherent similarity of constitution of the blood corpuscles. Nuttall (Blood Immunity and Relationship. Precipitin Tests. Cambridge Univ. Press, 1904) has shown that goat serum when treated with sheep antiserum gave a very marked precipitin reaction. This would indicate a rather close blood relationship between goat and sheep. Guinea pig and rabbit sera gave no reaction with the sheep anti-serum, but these animals are farther removed zoölogically in their relationship to sheep. The hæmolytic test as regards the weak complementing (toxic) ability of fresh serum upon specific immune body of closely related species might serve as an aid in distinguishing blood relationship in certain cases.* Noguchi found that goat serum was very efficient as a complement with anti-human rabbit immune body. In neither case was the combining ability of the sheep nor goat complementing serum determined; therefore, we cannot definitely say which property of the complement was responsible for the results. As evident, a number of factors influence hæmolysis; some of these are known, while perhaps there are others which have not been recognized as yet. Certain of these factors may act favorably; others may exert the opposite effect. The materials (antigens) injected in the preparation of a hæmolytic immune body by animal inoculation

* A number of exceptions must be made, as the normal serum from some species shows very poor complementing action upon most immune bodies, or upon particular ones.

are exceedingly complex in character. Moreover, the chemical composition is not absolutely constant. Consequently the anti-products elaborated by the body cells must be heterogeneous, with the specific hæmolysins predominating, even in the same species. Different animal species vary one from the other in the ability of cell reaction. Therefore, we should expect that like immune sera from different species would vary in their hæmolytic reaction characteristics (within certain limits) as frequently occurs. As regards the blood cells themselves which are used in the test, it must be recognized that a homologous (or even from a closely related species) complementing serum does not exert marked toxic action, although it may combine with the sensitized blood cells. Apparently this is a factor in the protective action on the part of the body against its own possible hæmolytic products, as has been pointed out by Ehrlich. The question of variation in structure of the active elements which make up a specific hæmolytic serum has been much discussed by different workers. Many of the conclusions are hypothetical and not fully proved. Some of the work would indicate that an immune serum is composed of slightly different types of similar bodies, which react differently with different active complements. The variation in the dosage ratio of immune body shown by the two series of tests (Table I) made with guinea pig and rabbit sera as complement may be in part explained by such a supposition. The complementing serum plays the predominant rôle in hæmolysis. It exerts a toxic or destructive action upon the cell under suitable conditions. Its lability makes it readily subject to functional change, or chemical alteration. We may consider a complementing serum to be composed of the active complement, inactive complementoid, and numerous intermediary products which fall in between these extremes, these products varying greatly as the serum approaches the complementoid state. Necessarily we have a very complicated product including these constituents alone. But there is evidence that serum also carries other products which exert an anti-complementary action, especially evident as the serum becomes aged. In view of the fact that relatively large volumes of serum must be used as complement, we are introducing most of our disturbing factors in this way. The factors which are associated more directly with one or the other of the three principal elements (blood cells, immune body, and complement) which enter the hæmolytic system, when combined in different ways, have an important bearing upon the resulting reaction. As previously pointed out, if the complement be decreased, an increase in immune body will compensate and give hæmolysis under certain conditions. Other factors, particularly of a physico-chemical nature, also are of extreme importance in the hæmolytic reaction. They will not be discussed in this connection, but elsewhere independently.

A PRACTICAL MODIFICATION OF THE WINSLOW DIRT FILTER

M. C. Schroeder, M. D.

The following apparatus is a modification and adaptation of the apparatus known as the Winslow Dirt Tester for the rapid determination of the amount of visible dirt in milk. This modification was designed primarily to overcome the difficulties which exist in testing the milk supply of New York City. These difficulties, stated briefly, are: First, those of transportation, owing to the distant and varied points from which New York receives its milk supply; second, the necessity of testing numerous samples of milk in a short space of time at their source in the country or upon its arrival at the wholesale plants in the city.

The original Winslow Dirt Tester, which was first used in Chicago, consists of a small unit (see illustration) and is composed of two cylinders made of copper, one within the other. The outer cylinder is 7⅜ inches long and 3⅜ inches wide; this cylinder has a narrow piece of tubing fitted into the lower portion, which is so arranged that it may be connected by means of rubber tubing with a hot water supply. In the upper portion of the cylinder is an opening providing an outflow for the water.

The inner cylinder is 7⅜ inches long and 2½ inches wide. At the bottom of this cylinder is an opening 1 inch in diameter. This opening is fitted on its outer side with a brass or copper ring surmounted by a smaller ring. There is also a removable cap with a detachable perforated brass disk. This cap is held in place by means of a copper wire. When the filter is to be used a disk made of absorbent cotton is placed within the cap. The inlet tube is attached by means of rubber tubing to a hot water supply and the space between the two cylinders is filled with water. A quart of milk is poured into the inner cylinder and is filtered through the cotton. After the milk has passed through the cotton is removed and the process repeated. This apparatus, while efficient, was not suited to the needs of New York City owing to the complications previously stated.

The following apparatus was therefore planned for the purpose of eliminating these difficulties. It consisted of four parts: First, the individual tester; second, the hot water tank or jacket; third, the lamp; fourth, the reservoir or carrying case.

The individual tester consists of a cylinder 7½ inches long, 1¾ inches wide at top and 1¼ inches at bottom (see Fig. A). It is made of spun copper brazed, and is fitted at the bottom with a skeleton brass cap fitted with a shoulder. Attached to the cylinder is a copper catch holding a bent wire which clasps over the cap and holds it in place; inside of these skeleton caps are small removable perforated brass disks. The small disks of cotton are bought wholesale, and when the milk is to be tested the cotton is placed in the cap on the disk and the cap is slipped into place.

The Water Jacket.—This water jacket (see Fig. B) was found necessary owing to the fact that cold milk or milk with ice in it does not filter rapidly. At the same time, it permits a separation of the cream, which, being finally deposited upon the filter, covers over the sediment and does not permit a true estimation of the amount of visible dirt present in the milk.

The hot water jacket is a covered, box-shaped tank having 10 cone-shaped, cylindrical openings passing through its entire depth for the reception of the individual testers, and is provided with a water inlet, situated on its upper surface. A water outlet closed with a screw cap or cork is placed in the left side wall and a steam vent is located in the upper side wall.

The jacket is 18 inches long, 6½ inches wide at the bottom and 15 inches long; 6½ inches wide at the top; it is 7½ inches high and has a

covered extension 2¼ inches high, 3 inches long, 6½ inches wide, for the direct application of heat. On the under surface of the hot water tank is a movable brass bar, 9 inches long. When the apparatus is to be used this bar is placed in a transverse position and acts as a support for the tank when the tank is placed above the reservoir.

Lamp for Use with the Tester.—The alcohol lamp designed for this tester was specially constructed for the purpose of giving the greatest amount of heat it was possible to produce. It consists of a powerful burner, regulated by a screw, so that the flame may be raised or lowered, and a reservoir, 6 inches long, 2¾ inches wide, 2 inches high, for holding the denatured alcohol. This lamp will maintain the temperature of the water in the jacket at near the boiling point, and will burn 2 to 3 hours without refilling. The reservoir is fitted at each end with a shoulder piece, 2¾ inches wide, 6 inches high, which has a turned edge upon its upper side. This edge slips over a corresponding ridge on the extension and holds the lamp in place when the apparatus is in use.

Carrying Case or Reservoir.—It was found that the most convenient case (see Fig. C) for transportation was one capable of accommodating 10 individual filters, together with the water jacket and lamp. The case was accordingly made of light copper, and had the following dimensions: Length, 18½ inches; height, 10¼ inches; width, 7 inches. The cover was hinged and closed anteriorly with a copper wire bolt, as shown in the illustration. For convenience in carrying a leather handle was attached to the cover by reinforced metal strips on the interior of the cover. The case was also planned to act as a reservoir for the milk which was being passed through the filters. When the reservoir is filled, the milk may be drawn off through the small detachable faucet shown in the illustration directly into a milk can. This milk can may be iced if the season requires it by standing it in a box of ice. In this way any loss of milk is obviated and the milk is not injured at all by the process, reaching the consumers a little cleaner for having undergone the filter test.

In testing out this apparatus at the creameries we went provided with a quart dipper and two one-quart measures; we also carried a number of 1½-inch square cards made of blotting paper. These were for the purpose of enabling us to fix the identification of the milk which is being tested.

When the apparatus is to be used the water tank is filled with hot water and by means of the brass bar adjusted over the reservoir. The lamp is filled with alcohol and placed under the extension and lighted.

The faucet is attached to the reservoir and an empty can is placed underneath the faucet. When the milk is received at the platform the inspector stirs up the milk in the can thoroughly; dips out one quart, writes the number or name of the farmer upon a small white square, placing it between the individual tester and the cylinder in the water tank, and pours the milk through the tester. Owing to the heat provided by the hot water tank, the milk passes rapidly through the strainers. After the milk has been filtered the cottons are removed and placed upon the square blotters, which bear the name or the number of the dairyman. These filter cottons are allowed to drain and are thus ready for immediate inspection by the dairymen. If it is desired to forward these samples to the laboratory for grading, the cottons are allowed to dry and then carefully packed and forwarded to the city.

If a permanent record of these filter-cottons is to be kept at the creamery, it is advisable to take a piece of board and cover it with zinc or glass, ruling upon its surface squares of at least two inches diameter. In these squares the farmers' names may be placed so that when the filter-cottons are removed from the filters they may be immediately placed on the squares; and thus identified, kept until investigation can be made concerning the conditions under which this milk was produced.

The standard of dirt which the Department expects to adopt consists of four grades: slight, medium, maximum and excessive. It has not been decided whether the cottons are to be forwarded to the laboratory for



FIG. A.—Individual Tester.

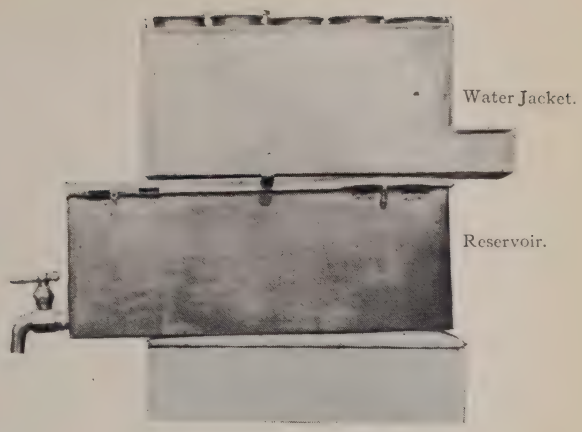
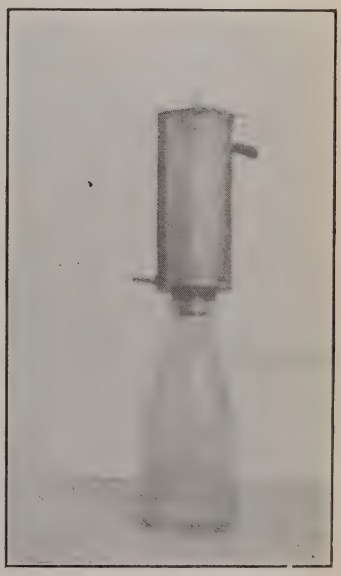


FIG. B.—Outfit ready for use.



FIG. C.—Outfit ready for Transportation.



Winslow Filter.

judgment or left to the judgment of the inspector. If they are to be left to the judgment of the inspectors, each inspector will be provided with a set of sample cottons, showing the grades of dirt mentioned. The maximum amount permitted will be that of the German Government, namely, 10 milligrams of dirt to every quart of milk.

This apparatus has been tested in a practical manner in a number of creameries, and it was found that very little inconvenience was caused to the creamery operators.

If the creamery is a large one, 75 to 200 patrons, two men are required to run the test, so that the milk may not be delayed in shipment; one man to take the samples; the second man to run the filter. In this manner the milk may be handled as fast as it is brought in by the dairymen. Special investigations were also made of those dairies from which particularly bad sediments were obtained. It was found that with few exceptions the bacteriological count and the sediment test tallied with the improper condition under which the milk was produced. Further work is being done along these lines. In closing I wish to express my thanks to Dr. W. H. Park, General Director of the Division of Laboratories, for the many helpful and practical suggestions which have made this study possible.

A STUDY OF THE BACTERIOLOGICAL AND SANITARY CONDITION OF THE MILK SUPPLY OF NEW YORK CITY

M. C. Schroeder

The following study of the bacteriological and sanitary condition of the milk supply of New York City was undertaken at the request of Dr. W. H. Park, general director of the Division of Laboratories, and was carried on and completed under his direct supervision.

The milk samples which form the basis of this study were taken by the members of the bacteriological squad and comprised samples of both city and country milk. Owing to the distances from New York City at which the creameries were located and the difficulties of transportation these men had to be provided with the simplest kind of an outfit for taking these samples. This outfit (see illustration a) consists of the following articles: an aluminum pipette 21 inches long and ¼ inch in diameter; a screw-necked vial 3 inches long and ⅞ of an inch in diameter, with an aluminum cap bearing a serial number; a round tin box 5 inches in diameter and 3¼ inches high to contain the sample bottles; two sizes of copper pipette holders of the round type; (*a*) holder 22 inches long, 2 inches in diameter, to hold 40 pipettes; (*b*) holder 22 inches long, 1 inch in diameter, to hold 10 pipettes. The pipettes, bottles, and cans are thoroughly sterilized on two consecutive days and then shipped once a week to the country inspectors.

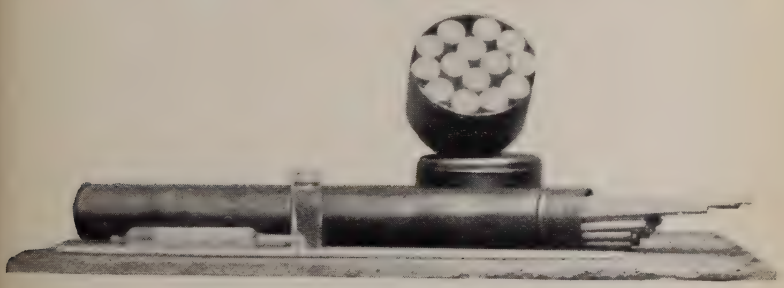


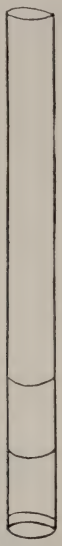
Illustration a.

The members of the bacteriological squad who take city samples carry pipettes, holders, and bottles of the same type, but in addition are provided with leather bags 18 inches long, log-cabin type, lined with aluminum and fitted with trays for holding the milk samples, and ice to keep them cold while being gathered.

Method of taking samples in the country.—The samples are taken by means of the aluminum pipettes before described, placed in the one-ounce viáls, packed in the round tins, placed in an old milk can, and thoroughly surrounded with ice. A hole is made in the bottom of the can so as to provide for drainage. The can is then wired, labeled, and shipped on the milk freight train together with the milk from which the samples were taken. Upon their arrival at the city terminal the samples are at once collected by an inspector who ices them again thoroughly and brings them to the laboratory, where he checks up the samples and writes his report. He also frequently takes control samples from the regular milk shipments. The method of taking the samples in the city is practically the same, except that the inspectors use the bags described and bring the samples directly to the laboratory.

Methods employed at the laboratory.—(*a*) All glassware used in milk work is sterilized for an hour at 160° F. on two consecutive days. The

dilution bottles are of the square type and are graduated at 50 c. c. and 100 c. c., respectively. The water used for dilutions is ordinary tap water filtered through a Berkefeld filter and sterilized by boiling on two consecutive days in a specially constructed heater from which it is filled directly into the bottles and used without resterilizing. The pipettes used are of two kinds, the ordinary volumetric pipette graduated to 1-100 c. c. and a short pipette 5 inches long and ¼ inch in diameter with three markings, ½ c.c.-1 c. c. (see illustration b). We are also using what we term the ring method.



Special pipette in use.

Illustration b.

Ring method.—It was found after a number of experiments that a small ⅛-inch No. 20 japanned curtain ring would take up within 5 per cent. of 0.01 c. c. of milk, and that, by dropping this ring full of milk directly into the melted agar tube, we could eliminate one or more of the dilution bottles and thus save labor and a possible source of error. The rings are dipped into the milk by means of a platinum hook and dropped directly into about 8 c. c. of nutrient agar held in the tube.

Method of plating and counting.—Each inoculated tube is shaken 10 times and plated. The Petri dishes, after marking, are packed into paper-lined crates holding 60 or more plates and incubated at a temperature of 37° C. for 48 hours. They are then examined and counted and the count placed directly upon the plates. From these plates the counts are entered upon the bacteriological cards sent to us by the inspectors.

Method of recording data obtained from cards.—The items of information concerning the samples taken in the country are the location of the creamery, its owner, the name of the dairyman, the time the sample was taken, the temperature and the quality of the milk, the manner of cooling, number of samples, and the bacteriological result (see Fig. 1). The city card varies somewhat, as shown in the illustration (Fig. 2). In the laboratory the results from the creameries are recorded on the ledger sheet shown in Fig. 3; the sheet gives the name of the owner, name and location of creameries controlled by him, the kinds of milk shipped, bacterio-

logical counts, the temperature of samples, the manner of cooling the milk, the result of the dirt test, and other facts bearing upon the bacteriological results. These sheets are kept in ledger form and a monthly statement is drawn. The bacteriological results from the samples taken in the city are tabulated in a similar manner.

Form No. 86 F-1911 1738–11 (B) 1500

DEPARTMENT OF HEALTH
THE CITY OF NEW YORK

CREAMERY AT..... Average Count.....

Operator.....

Date..... 191 Air Temp. ..... ° Fahr.

NAME	TIME	ICE	MILK	TEMP.	SAMPLE No.	BACTERIAL COUNT

Signed ..... Bacteriologist Signed ..... Inspector.

(Size of card, 5×8.)

FIG. 1.

Analysis of samples.—The total number of bacteriological analyses made was 61,142. The various sources are best shown in Table 1.

TABLE 1.

Source	No. of Samples
Country creameries	22,761
Pasteurizing plants	5,049
Stores	19,876
Wagons	6,132
Hospitals	5,406
Infant health stations	504
Special examinations	1,414
	61,142

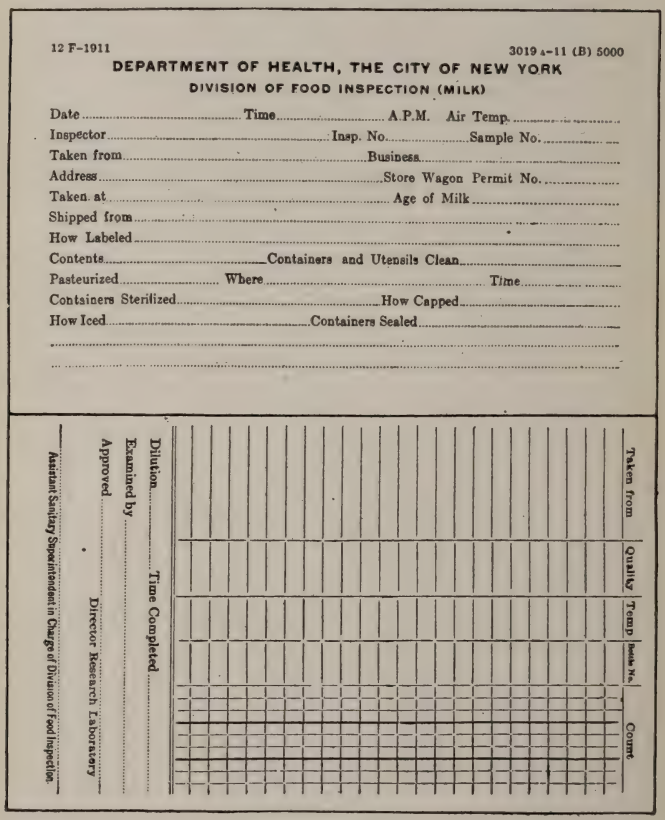
For the purpose of this study there were taken 20,334 samples of country milk and 1,950 samples of milk as delivered in the city. These samples of country milk represent the milk from 432 creameries situated as previously stated. The creameries are controlled by 120 of the large dealers who supply New York City with milk.

Analysis of country milk.—A study of the bacteriological findings as recorded in Table 2 shows that of the 20,334 samples taken for bacteriological examinations, 7,756, or 38.14 per cent., contained less than 10,000

bacteria per c. c.; 6,633, or 32.61 per cent., contained from 10,000 to 50,000 bacteria per c.c.; 2,403, or 11.81 per cent., contained from 50,000 to 100,000 bacteria per c.c.; 2,723, or 13.39 per cent., contained from 100,000 to 1,000,000 bacteria per c.c.; 819, or 4.02 per cent., contained 1,000,000 or over bacteria per c.c.

Adding together the number of samples which contained less than 50,000 bacteria per c. c., it will be found that 14,389, or 70.75 per cent. of all samples analyzed, fall into this class.

Contrasting the highest with the lowest percentages of samples of each group, it will be found that in the division of less than 10,000


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DEPARTMENT OF HEALTH, THE CITY OF NEW YORK

DIVISION OF FOOD INSPECTION (MILK)

Date ..... Time ..... A.P.M. Air Temp. .....

Inspector ..... Insp. No. ..... Sample No. .....

Taken from ..... Business .....

Address ..... Store Wagon Permit No. .....

Taken at ..... Age of Milk .....

Shipped from .....

How Labeled .....

Contents ..... Containers and Utensils Clean .....

Pasteurized ..... Where ..... Time .....

Containers Sterilized ..... How Capped .....

How Iced ..... Containers Sealed .....

Dilution ..... Time Completed .....

Examined by .....

Approved .....

Director Research Laboratory

Assistant Sanitary Superintendent in Charge of Division of Food Inspection

Taken from

Quality

Temp

Sediment No.

Count

(Size of card, 3×5.)

FIG. 2.

per c. c. April ranks highest, having 673 samples, or 59 per cent., out of a total of 1,140 samples; August lowest, with 332 samples, or 23.8 per cent., out of a total of 1,393.

In the 10,000-50,000 group February ranks highest, having 613 samples, or 41.68 per cent., out of a total of 1,466 samples; August lowest, with 340 samples, or 24.38 per cent., out of a total of 1,393.

In the 50,000-100,000 group July ranks highest, having 132 samples, or 15.84 per cent., out of a total of 830 samples; April lowest, with 78 samples, or 6.78 per cent., out of a total of 1,140.

In the 100,000-1,000,000 group September ranks highest, having 525 samples, or 24.39 per cent., out of a total of 2,152 samples; March lowest, with 39 samples, or 3.13 per cent., out of a total of 1,241.

In the group containing 1,000,000 or over per c. c. August ranks highest, having 221 samples, or 15.85 per cent., out of a total of 1,393

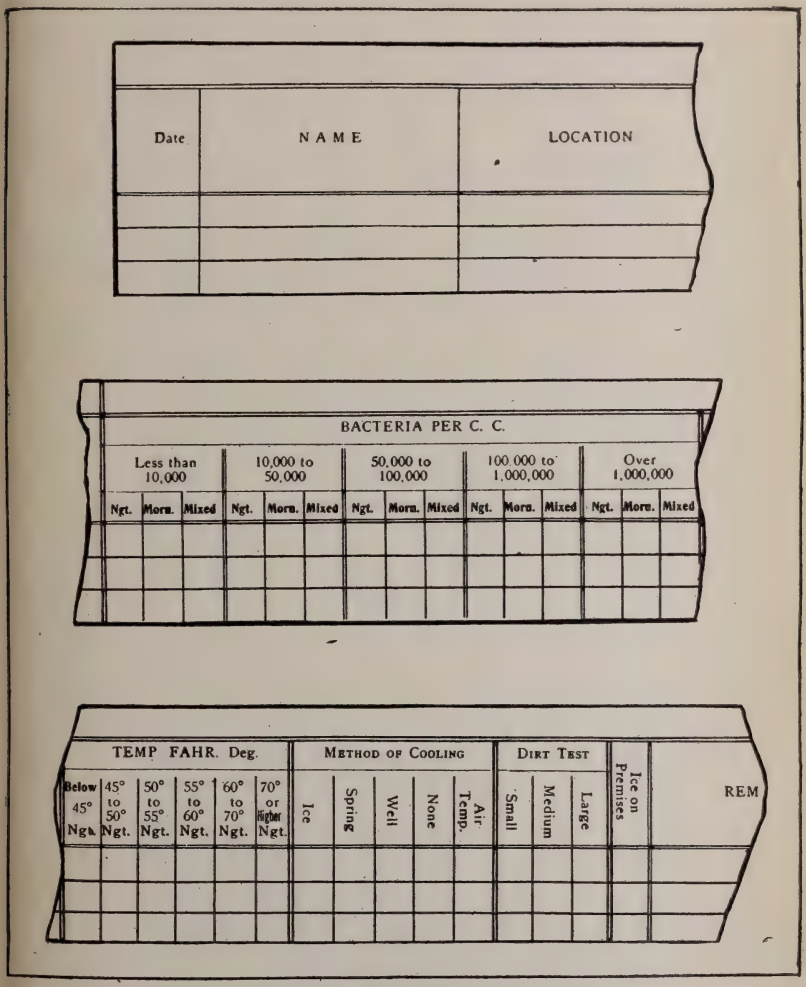


FIG. 3.—Headings on ledger sheet.

samples; November lowest, with 6 samples, or 0.23 per cent., out of a total of 2,514.

Temperature of milk at time of delivery.—The temperatures of the milk from which those samples were taken are shown in Table 3.

Owing to the extreme haste with which these samples had to be taken at times, and other adverse circumstances, it was not always possible to procure the temperatures of the milk at the time it was sampled. We

TABLE 2.

ANALYSIS OF BACTERIOLOGICAL COUNTS.

	TOTAL OF SAMPLES	BACTERIA PER C. C.									
		Less than 10,000		10,000 to 50,000		50,000 to 100,000		100,000 to 1,000,000		1,000,000 or over	
		No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage
January....	1,274	579	45.44	472	37.	128	10.	91	7.14	4	.3
February...	1,466	578	39.42	613	41.68	169	11.49	100	6.8	6	.4
March.....	1,241	682	54.90	421	33.89	90	7.24	39	3.13	9	.7
April.....	1,140	673	59.	289	25.14	78	6.78	94	8.17	6	.52
May.....	2,097	874	41.95	580	27.84	303	14.54	293	14.06	47	2.25
June.....	2,099	621	29.58	689	32.82	206	9.81	410	19.53	173	8.24
July.....	830	299	35.88	239	28.68	132	15.84	130	15.60	30	3.60
August....	1,393	332	23.81	340	24.38	181	13.18	319	22.67	221	15.85
September .	2,152	550	25.55	533	24.76	298	13.84	525	24.39	246	11.43
October....	2,802	1,135	39.72	934	32.59	315	11.02	358	12.53	60	2.1
November .	2,514	988	39.29	1,032	41.05	308	12.25	180	7.15	6	.23
December..	1,326	445	33.55	491	37.02	195	14.71	184	13.87	11	.82
	20,334	7,756	38.14	6,633	32.61	2,403	11.81	2,723	13.39	819	4.02

were fortunate enough, however, to obtain the temperature of 18,698 specimens of milk, and the percentages shown in Table 3 are based upon these figures. This table also shows the number and percentage of samples in the divisions adopted, namely, below 45° F., 45-50°, 50-55°, 55-60°, 60-70°, and 70° F. and over. They are as follows:

Below 45° F. there were 3,767 samples, or 20.1 per cent., out of a total of 18,698.

45 to 50° F. there were 3,568 samples, or 19 per cent., out of a total of 18,698.

50 to 55° F. there were 3,945 samples, or 21 per cent., out of a total of 18,698.

55 to 60° F. there were 3,603 samples, or 19.2 per cent., out of a total of 18,698.

60 to 70° F. there were 3,141 samples, or 16.7 per cent., out of a total of 18,698.

70° F. or over there were 674 samples, or 3.6 per cent., out of a total of 18,698.

Contrasting the highest with the lowest percentage of samples having a temperature of less than 50° F., it will be found that in the division of temperature less than 50° F. February ranks highest with 771 samples, or 72 per cent., out of a total of 1,063 samples; August lowest, with 55 samples, or 4.6 per cent., out of a total of 1,171.

In the division of temperatures from 50 to 60° F. June ranks highest, with 1,069 samples, or 54.8 per cent., out of a total of 1,969 samples; November lowest, with 570 samples, or 23 per cent., out of a total of 2,438 samples.

In the division of temperatures from 60 to 70° F. August ranks highest, with 604 samples, or 51 per cent., out of a total of 1,171 samples; March lowest, with 34 samples, or 2.7 per cent., out of a total of 1,241 samples.

TABLE 3.

TEMPERATURE OF MILK AS RECEIVED AT CREAMERIES

MONTHS 1911	TEMPERATURE FAHRENHEIT													
	Total Samples	Temperatures Stated in	Temperatures Not Stated in	Less than 45°		45° to 50°		50° to 55°		55° to 60°		60° to 70°		70° and Over	
				No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
January	1,274	987	287	408	41.33	269	27.23	179	18.11	80	8.1	42	4.25	9	0.9
February	1,466	1,063	403	521	49.01	250	23.51	123	11.57	82	7.7	47	4.42	40	3.75
March	1,241	1,224	17	397	32.46	449	36.6	251	20.5	86	7.0	34	2.7	7	.57
April	1,140	1,024	116	315	30.71	337	32.8	225	21.9	91	8.8	37	3.6	19	1.8
May	2,097	2,034	63	105	5.16	265	13.02	441	21.68	625	30.72	549	26.49	49	2.40
June	2,099	1,969	130	27	1.37	121	6.1	533	27.06	536	27.22	615	31.23	137	6.95
July	830	721	109	15	1.98	31	4.2	137	19.0	216	29.9	168	23.3	154	21.3
August	1,393	1,171	222	14	1.19	41	3.5	121	10.3	271	23.1	604	51.5	120	10.2
September	2,152	2,056	96	95	4.62	154	7.49	391	19.01	697	33.90	640	31.12	79	3.84
October	2,802	2,690	112	307	11.41	625	23.23	803	29.85	644	23.94	273	10.14	38	1.41
November	2,514	2,438	76	1,047	42.92	745	30.5	396	16.2	174	7.1	70	2.8	6	.24
December	1,326	1,321	5	516	39.0	281	21.2	345	26.0	101	7.6	62	4.6	16	1.2
	20,334	18,698	1,636	3,767	20.13	3,568	19.08	3,945	21.09	3,603	19.26	3,141	16.74	674	3.6

In the last division, 70° F. or over, July ranks highest with 154 samples, or 21 per cent., out of a total of 721 samples.

Method of cooling.—The data which were gathered by the inspectors concerning the method of cooling employed by the dairymen to cool their milk are given in Table 4. It will be noticed that the same discrepancy

TABLE 4.

METHOD OF COOLING.

MONTH OF YEAR	NUMBER OF SAMPLES	SAMPLES FOR WHICH COOLING METHOD WAS NOT STATED		SAMPLES FOR WHICH COOLING METHOD WAS STATED		SAMPLES FOR WHICH COOLING METHOD WAS ICE		SAMPLES FOR WHICH COOLING METHOD WAS NOT ICE	
		No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
January....	1,274	454	35.63	820	64.35	422	51.46	398	48.53
February...	1,466	851	58.04	615	41.95	371	60.32	244	39.67
March.....	1,241	290	23.36	951	76.63	449	47.21	502	52.78
April.....	1,140	69	6.05	1,071	93.94	659	61.53	412	38.47
May.....	2,097	237	11.47	1,860	88.53	1,345	72.31	515	27.69
June.....	2,099	635	30.22	1,464	69.74	812	55.46	652	44.53
July.....	830	130	15.66	700	84.33	354	50.57	346	49.42
August....	1,393	224	16.08	1,169	83.91	583	49.87	586	50.12
September..	2,152	358	16.62	1,794	83.31	525	28.71	1,269	71.23
October....	2,802	212	8.63	2,560	91.34	621	24.25	1,939	75.74
November..	2,514	263	10.46	2,251	89.36	499	21.95	1,752	77.83
December..	1,326	106	8.25	1,220	91.50	268	21.94	952	78.03
	20,334	3,859	18.97	16,475	81.02	6,908	41.93	9,567	58.06

which has already been spoken of exists between the total number of samples taken and the number in which data were obtainable. The total number of samples in which the cooling method was stated was 16,475, or 81.02 per cent.; the number in which the method of cooling was not stated was 3,859, or 18.97 per cent.

Of those in which the method of cooling was stated, ice was used in 6,908 instances, or 41.93 per cent., and was not used in 9,567 instances, or 58.06 per cent. We also found that the number and percentage of samples where ice was used varied in the different quarters of the year. This factor is also influenced by the character and location of the creameries, the dairymen drawing to the better-class creameries using ice more frequently than those who drew to the poorer class. Grouping the results obtained into the four quarters of the year gives us the following summary:

	Total Number of Samples Delivered at Creameries	Total Number in Which Cooling Was Stated	Number Using Ice	Percentage
1st quarter.....	3,981	2,386	1,242	52
2d "	5,336	4,395	2,816	64
3d "	4,375	3,663	1,462	39
4th "	6,642	6,031	1,388	22
	20,334	16,475	6,908	41

It will be seen from these summaries how comparatively few dairymen use ice in cooling their milk. It is of interest to note that in July 50 per cent. of the milk brought in was cooled by ice, 61 per cent. at a temperature of 50° F. or under, and 64 per cent. of the milk contained less than 50,000 bacteria per c. c. The greater part of the samples taken in July were of those known as morning's milk. This milk is usually brought to the creameries without cooling, therefore the records of the temperatures of the milk in July were high and the bacteriological counts low.

The preceding analysis was based upon the total number of samples taken and their relation to each other as a whole. In the following analysis (Table 5) the milk is divided into the three grades in which it is delivered to the creameries by the dairymen; namely, night's, morning's, and mixed milk. It therefore shows in addition the number of samples of each grade taken, together with the number and percentage of samples falling into each one of the five groups of bacteriological counts. It also shows the temperatures of the samples taken from these three grades of milk as they were delivered at the creameries, and the number and percentage of the samples in each of the six divisions we have adopted as a standard of temperatures. It also gives a detailed statement of the methods of cooling employed by the dairymen. Taking up the different groups of bacteriological counts we find that the morning's milk had the highest percentage of low counts, 82.19 per cent. having less than 50,000 bacteria per c. c., and the night's milk had the lowest percentage of samples in that group, namely, 64.8 per cent. A brief summary of these percentages is given below:

BACTERIAL COUNTS PER c. c.

Type of Milk	Total Number of Samples	Less than 50,000		50,000 to 100,000		100,000 to 1,000,000		1,000,000 to 100,000,000	
		Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage
Night's...	9,885	6,477	64.8	1,244	12.45	1,622	16.2	542	6.4
Morning's.	1,202	988	82.19	111	9.23	89	7.4	14	1.6
Mixed....	9,247	6,924	74.	1,048	11.5	1,012	11.11	263	2.8
		14,389	70.76	2,403	11.81	2,723	13.39	819.	4.02

Night's milk.—Considering the preceding summary it will be noticed that, while 64 per cent. of the samples are under 50,000, 34 per cent. contained 50,000 or over per c. c., notwithstanding the fact that the largest number of samples were taken during the cold months, when the atmospheric temperature would naturally have some effect in reducing the bacterial content and holding bacterial growth in check. This comparatively large percentage of bacteria may be ascribed to either one or both of two factors: increased contamination of milk due to the fact that the cows are housed and not kept as clean as in the summer time, and use of utensils which were not clean.

While comparatively few samples of morning's milk were sent to the laboratory for analysis, it is of interest to note that 46.8 per cent. had less than 10,000 bacteria per c. c. and 82 per cent. of the samples contained less than 50,000 bacteria per c. c. This low bacterial content held its own during the months of April, May, June and July, when the percentages

were respectively 81, 75, 79, 87 per cent. of the total number of samples taken of morning's milk for those months. These results bear out the statement made above concerning time as a factor as well as the extra contamination of milk during the winter months, and the effect of the unclean utensils. Most of the morning's milk was delivered within two hours after milking. This milk is an average between the older half and the fresher half of the day's milk. The longer the milk is kept the greater the effect due to the three factors stated; namely, by increased contamination due to the unclean condition of the cattle, unclean utensils, and lack of icing. Owing to the fact that it approximates closely the grade of milk delivered to city consumers for the entire year, a detailed study of the bacterial count of the mixed milk was made. The bacteriological curve obtained is shown in Chart 1. A comparison of the chart with the table gives us the percentages shown. These are briefly summarized as follows:

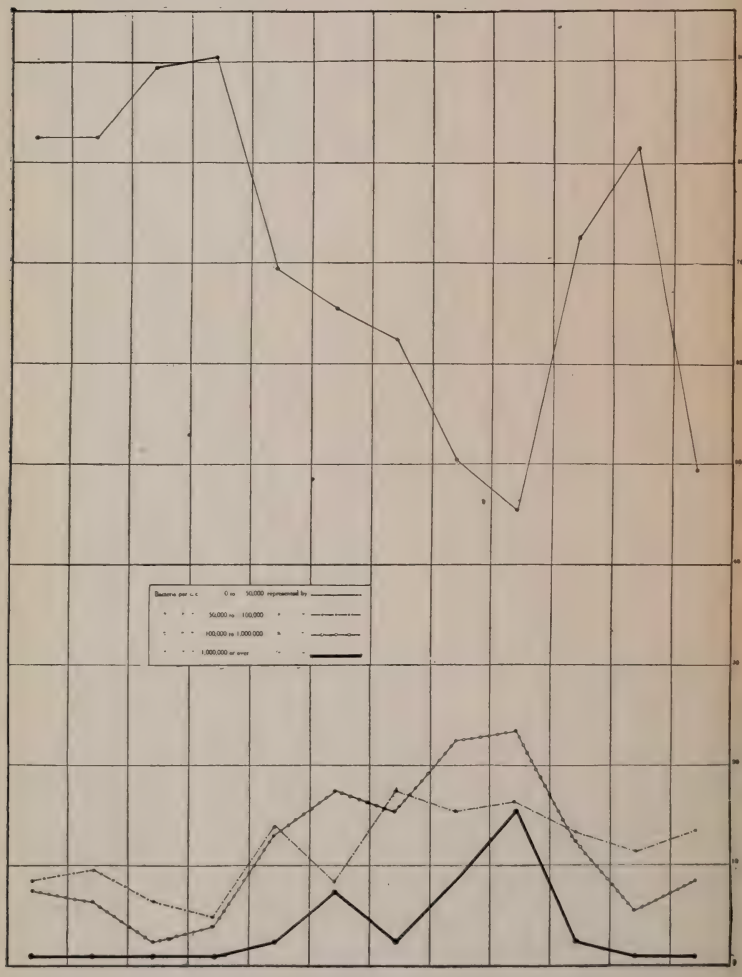


Chart 1.

TABLE 6.

Quarterly Statement	Number of Samples Taken for Examination	Number of Dealers from Whom Samples Were Taken	Number of Creameries Represented	Unknown Sources	Origin of Milk as Stated on Labels				Age of Milk								Method of Cooling				Temperatures										Bacteriological Count									
					Stated		Not Stated		From 24 to 36 Hours		From 36 to 48 Hours		48 Hours and Over		Stated		Not Stated		Under 45° F.		From 45° to 50° F.		From 50° to 55° F.		From 55° to 60° F.		From 60° to 70° F.		Less than 10,000		From 10,000 to 50,000		From 50,000 to 100,000		From 100,000 to 1,000,000		Over 1,000,000	
					No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage	No.	Per-centage
January -																																						
Raw, cans	100	26	26	9	85	85	65	65	35	35	55	85			10	15	28	28	72	72	89	89	1	1	10	10					6	6	17	17	13	13	37	37	27	27
" bottles	100	23	23	1	98	98	73	73	27	27	45	62	25	34	3	4	95	95	5	5	82	82	13	13	5	5					9	9	25	25	9	9	11	11	16	16
Past., cans	100	2	2	6	35	35	90	90	10	10	56	62	21	23	13	15	100	100			92	92	8	8							21	21	13	13	5	5	10	10	1	1
" bottles	100	11	6	13	31	31	84	84	16	16	39	47	23	27	22	26	100	100			90	90					4	4	6	6	19	19	28	28	19	19	4	4		
Heated, bottles	100	11	7	13	21	24	48	48	52	52	30	62	12	25	6	13	97	97	3	3	77	77	21	21	2	2					16	16	29	29	7	7	25	25	24	24
April -																																						
Raw, cans	100	12	56	2	96	96	94	94	6	6	63	67	8	9	23	24	64	64	36	36	78	78	11	11	11	11					7	7	19	16	17	17	15	15	21	21
" bottles	100	26	22	3	88	88	100	100			78	78	18	18	4	4	100	100			79	79	17	17	4	4					7	7	17	17	16	16	19	19	14	14
Past., cans	100	3	5	6	39	39	67	67	33	33	23	34	36	54	8	12	85	85	15	15	41	41	59	59	9	9					55	85	33	33	4	4	2	2	1	1
" bottles	100	11	18	14	45	45	94	94	6	6	38	40	38	49	18	20	94	94	6	6	62	62	24	24	14	14					19	19	17	17	16	16	13	14	3	3
Heated, bottles	50	9	9	8	22	44	31	62	19	38	8	26	14	45	9	29	50	100			26	52	7	14	11	22	6	12			5	10	16	32	8	16	20	10	5	10
July -																																						
Raw, cans	100	30	86		100	100	82	82	18	18	70	85			12	15	91	91	9	9	26	26	45	45	17	17	8	8	4	4	7	7	3	3	3	3	19	19	17	17
" bottles	100	18	21		100	100	88	88	12	12	88	100					100	100			56	56	20	20	21	21	3	3					15	15	8	8	31	31	23	23
Past., cans	100	6	4	19	17	17	21	21	79	79	17	81	4	19			99	99	1	1	23	23	28	28	39	39	5	5	5	5	42	42	26	26	12	12	17	17	3	3
" bottles	100	18	1	13	20	20	50	50	50	50	7	14	27	54	16	32	100	100			37	37	19	19	30	30	14	14			14	14	35	35	29	29	11	11		
Heated, bottles	50	4	1	12	5	10	19	38	31	62			19	100			50	100			6	12	25	50	16	32	3	6			9	18	15	30	7	14	9	18		
October																																						
Raw, cans	100	38	35	3	97	97	91	91	9	9	65	71	17	19	9	9	99	99	1	1	32	32	57	57	11	11														
" bottles	100	24	27	2	91	91	97	97	3	3	85	88	6	6	6	6	100	100			77	77	13	13	10	10					1	1	11	11	12	12	13	14	33	33
Past., cans	100	6	4	12	22	22	28	28	72	72	5	18	22	78	1	4	96	96	4	4	87	87	5	5	8	8					6	6	10	10	8	8	6	6	29	29
" bottles	100	13	3	11	11	11	33	33	67	67	8	24	22	67	3	9	90	90	10	10	62	62	21	21	17	17					65	65	18	18	11	11	4	4	3	3
Heated, bottles	50	8	1	9	2	4	27	54	23	46	4	15	23	85			50	100			33	66	12	24	5	10					25	25	9	9	12	12	2	2	3	3
Heated cans, for 1911	100	7	6	17	37	37	55	55	45	45	31	56	10	35	5	9	90	90	10	10	51	51	7	7	41	41	1	1			20	20	18	36	8	16	14	28		
																															23	23	39	39	11	11	12	12	1	1

Months	Total Number of Samples of Mixed Milk		Less Than 50,000 Bacteria per c. c.		50,000 to 100,000 Bacteria per c. c.		100,000 to 1,000,000 Bacteria per c. c.		1,000,000 or over Bacteria per c. c.	
	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage
January...	912	100	757	82.96	81	8.87	72	7.89	2	.10
February..	961	"	796	82.83	93	9.67	66	6.86	6	.62
March....	900	"	810	90.00	60	6.6	27	2.9	3	.33
April.....	467	"	423	90.57	22	4.7	21	4.49	1	.21
May.....	1,257	"	877	69.68	175	13.82	172	13.58	33	2.6
June.....	931	"	613	65.73	84	8.98	166	17.76	68	7.27
July.....	429	"	268	62.47	83	17.33	68	15.64	10	2.33
August....	385	"	202	52.46	60	15.00	88	22.00	35	8.75
September.	518	"	234	45.17	84	16.37	120	23.39	80	15.59
October...	761	"	544	71.48	101	13.23	95	12.44	21	2.75
November.	1,223	"	1,010	82.5	138	11.17	74	5.99	1	.08
December.	503	"	390	77.53	67	13.32	43	8.54	3	.59

It will be observed that the percentage of milk containing less than 50,000 bacteria per c. c. drops steadily from its highest level of 91 per cent. in April until it reaches the lowest point, or 45 per cent., in September, and that the percentage of milk containing a million or more bacteria per c. c. follows the inverse curve and reaches its highest point in July. Table 5 also shows clearly that most of the samples were brought in at a temperature ranging from 50 to 60° F., and a considerable portion between 60 and 70° F.

Having thus briefly considered the bacteriological content of the milk as received at the creameries, together with a few of the factors influencing the same, it was thought best to consider also the milk as it was offered for sale in New York City, in order to ascertain: (1) what was the general bacteriological content of the milk sold; (2) the age of milk as offered for sale; (3) the relative bacteriological content of the raw milk sold in cans or bottles; of the pasteurized milk sold in cans or bottles, together with samples taken of the milk pasteurized by the flash process and known as heated milk; (4) some of the sanitary conditions surrounding its care and sale. For this purpose 1,950 samples were selected from the large number of samples examined. Four months of the year, January, April, July and October, were selected as representative types of the different seasons, and 100 samples of each month's supply of milk of the grades mentioned were chosen, except in the case of heated milk, where it was impossible to procure this number from different dealers. These numbers represent the milk supplied by 160 of the principal dealers, being carefully selected to represent as many sources and dealers as possible. The data procured are set forth in Table 6. Considering these data in detail, we find that the raw milk sold in cans and bottles, taken as a whole, gives the following results:

Months	Total Number of Samples	Less than 50,000 Bacteria per c. c.		50,000 to 100,000 Bacteria per c. c.		100,000 to 1,000,000 Bacteria per c. c.		1,000,000 or over Bacteria per c. c.	
		Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage
January	200	57	28.5	22	11.	78	40.53	43	20.5
April..	200	47	23.5	33	16.5	88	44.	36	18.
July...	200	25	12.5	11	5.5	94	47.	70	35.
October	200	28	14.	20	10.	80	40.	72	36.

Contrasting the above results with the bacteriological counts of the milk as delivered to the creameries in the country during these months, the following differences present themselves:

Months	Total Number of Samples	Less than 50,000 Bacteria per c. c.		50,000 to 100,000 Bacteria per c. c.		100,000 to 1,000,000 Bacteria per c. c.		1,000,000 Bacteria per c. c.	
		Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage
January									
Country..	1,274	1,051	82.4	128	10.	91	7.13	4	.3
City.....	200	57	28.5	22	10.5	78	39.	43	21.5
April									
Country..	1,140	962	84.3	78	6.7	94	8.1	6	.52
City.....	200	47	23.5	33	16.5	84	42.	36	18.
July									
Country..	830	538	64.	132	15.84	130	15.60	30	3.6
City.....	200	25	12.5	11	5.5	94	47.	70	35.
October									
Country..	2,802	2,069	73.84	315	11.02	358	12.5	60	2.1
City.....	200	28	14.	20	10.	80	40.	72	36.

The increase in the bacterial content of city milk over that of country milk is marked, and not wholly accounted for by the factor of time in allowing increase of bacteria. This may be partly accounted for by the fact that milk was frequently shipped in cans and bottles which were not clean and were poorly iced in transit.

Age of milk.—The following figures concerning the age of milk as sold in New York City are based upon the data placed upon the labels attached to the cans. These data do not, however, give the true age of the milk, as in numerous instances the milk is 12 or more hours old before it reaches the creamery, and then it may be held over at the creamery from 12 to 24 hours longer before it is placed upon the milk train for transmission to the city.

Months	Total Number of Samples	Number Age Not Stated	Number Age Stated	24–36 Hours Percentage	36–48 Hours Percentage	48 Hours and over Percentage
January...	200	62	138	72	19.	7.2
April.....	200	6	194	72	13.6	13.9
July.....	200	30	170	93	....	15.
October....	200	12	188	88	25.	16.

Based upon the above figures, it can be safely stated that 78 per cent. of the raw milk reaches New York and is sold from 24 to 36 hours after leaving the creamery; 10 per cent., 36 to 48 hours; 9 per cent., 48 hours and over, some of it being more than 72 hours old. The actual age would be about an additional 12 to 24 hours.

Temperature of raw milk as offered for sale in New York City.—The temperature of the milk while offered for sale, as shown by the following data, is interesting:

TEMPERATURES.

Months	Total Number of Samples	Under 45° F.		45–50° F.		50–55° F.		55–60° F.		60–70° F.	
		Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage	Number of Samples	Percentage
January..	200	171	85.5	14	7.	15	7.5	..	..	..	...
April....	200	157	78.5	28	14.	15	7.5	..	....	..	...
July.....	200	82	41.	65	32.5	38	19.	11	5.5	4	2.
October..	200	109	54.5	70	35.	21	10.5	..	....	..	...
		519	64.8	177	22.1	89	11.1	11	1.37	4	0.5

Taking the total number of samples of raw milk selected for the study, it will be seen that 64.8 per cent. of the milk examined was held in New York at a temperature of less than 45° F., 22 per cent. was found to be from 45 to 50° F., 11 per cent. was from 50 to 55° F., 1.37 per cent. from 55 to 60° F., and 0.5 per cent. 70° F. and over.

Comparison of bacterial counts of raw milk as sold in cans and bottles.—The figures upon which this comparison is based are given in Table 6. Taking the average bacterial count of the samples for each month, we find that with the exception of the samples examined in October there is not the great difference in the counts we had hoped to find in favor of bottled milk. A comparison of these counts is given below:

Month	Containers	Average Bacterial Counts
		per c. c.
January	Cans	318,500
	Bottles	317,450
April	Cans	295,450
	Bottles	169,050
July	Cans	510,000
	Bottles	289,000
October	Cans	380,160
	Bottles	43,230

Comparison showing the variation of the quantities of milk held at a temperature below 45° F.:

	January	April	July	October
	Percentage	Percentage	Percentage	Percentage
Cans	90	89	71	89
Bottles	95	96	76	90

It will be seen from the foregoing that the advantage of lower bacterial count and lower temperatures is slightly in favor of the milk sold in bottles.

Pasteurized milk sold in cans.—Pasteurized milk sold in cans is delivered, as a rule, to the hospitals, and therefore the output is fairly limited. It, however, represents the type of milk used by the hospitals during the past year.

PASTEURIZED MILK IN CANS.

Percentage of Age of Milk.

Months	Not Stated	Stated	24–36 Hours	36–48 Hours	48 Hours
January	10	90	62	23	15
April	33	67	34	54	12
July	79	21	81	19	..
October	72	28	18	78	4

From the above it will be noticed that in the month of July only 21 per cent. of the milk had the age stated on the tag.

Percentage of Temperatures.

Months	Below 45° F.	45–50° F.	50–55° F.	55–60° F.	60–70° F.
January	92	8	..	..	..
April	41	50	9	..	..
July	23	28	39	5	5
October	87	5	8	..	..

Percentage of Bacteriological Counts.

Months	Less than 50,000 Bacteria per c. c.	50,000 to 100,000 Bacteria per c. c.	100,000 to 1,000,000 Bacteria per c. c.	1,000,000 and over Bacteria per c. c.
January	84	5	10	1
April	88	4	7	1
July	68	12	17	3
October	83	11	3	3

PASTEURIZED MILK IN BOTTLES.

Percentage of Age of Milk.

Months	Not Stated	Stated	24–36 Hours	36–48 Hours	48 Hours and Over
January	16	84	47	27	26
April	6	94	40	40	20
July	50	50	14	54	32
October	67	33	24	67	9

Percentage of Temperatures.

Months	Under 45° F.	45–50° F.	50-55° F.	55–60° F.	60–70° F.
January	90	..	..	4	6
April	62	24	14	..	..
July	37	19	30	14	..
October	62	21	17	..	..

Percentage of Bacteriological Counts.

Months	Under 50,000 Bacteria per c. c.	50,000 to 100,000 Bacteria per c. c.	100,000 to 1,000,000 Bacteria per c. c.	1,000,000 and over Bacteria per c. c.
January	77	19	4	..
April	66	16	13	5
July	49	20	31	..
October	64	12	22	2

From the preceding tabulation it is seen that the bacterial counts in pasteurized milk are considerably less than in raw milk. Four factors—(1) improper pasteurization; (2) contamination of the milk through careless handling or unclean utensils; (3) holding too long, even up to 36 hours after pasteurization; (4) improper cooling methods—acting singly or together, account for the considerable percentage of persisting high counts.

The comparative bacteriological values of the four grades of milk sold in New York City are graphically shown in Chart 2 and accompany the data given below.

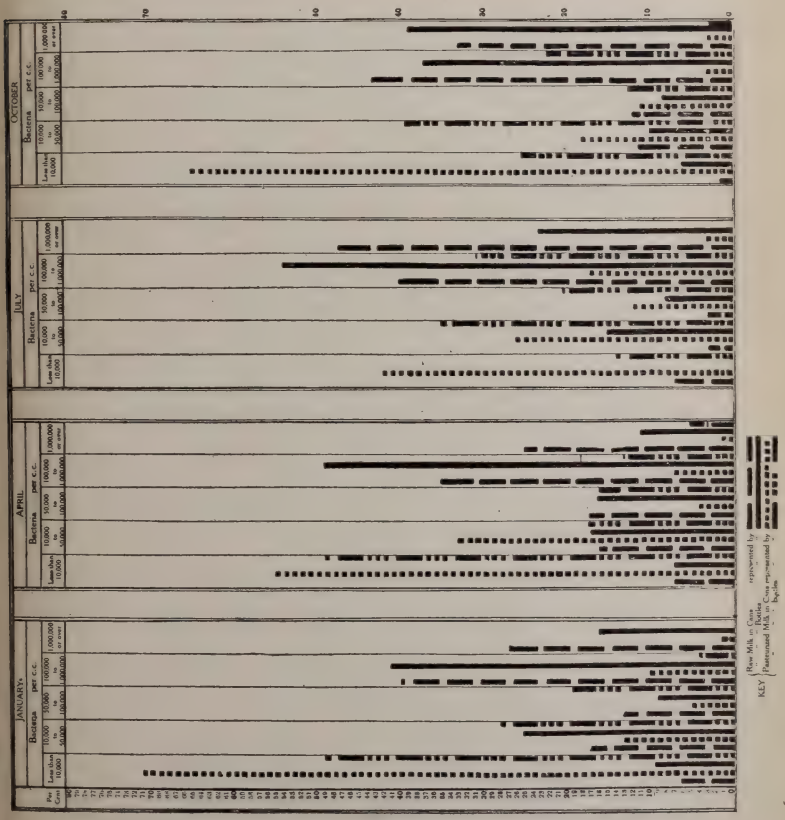


Chart 2.

Months	Type of Milk	Bacteriological Counts.				
		Less than 10,000 Bacteria per c. c.	10,000 to 50,000 Bacteria per c. c.	50,000 to 100,000 Bacteria per c. c.	100,000 to 1,000,000 Bacteria per c. c.	1,000,000 and over Bacteria per c. c.
		Percentage	Percentage	Percentage	Percentage	Percentage
January ..	Raw milk in cans	6	17	13	37	27
	Past. " " "	71	13	5	10	1
	Raw " " bottles	9	25	9	41	16
	Past. " " "	49	28	1	10	4
April.....	Raw milk in cans	7	16	17	35	25
	Past. " " "	55	33	4	7	1
	Raw " " bottles	7	17	16	49	11
	Past. " " "	49	17	16	13	5
July.....	Raw milk in cans	7	3	3	40	47
	Past. " " "	42	26	12	17	3
	Raw " " bottles	..	15	8	54	23
	Past. " " "	14	35	20	31	..
October...	Raw milk in cans	1	11	12	43	33
	Past. " " "	65	18	11	3	3
	Raw " " bottles	6	10	8	37	39
	Past. " " "	25	39	12	22	2

Heated milk in bottles.—As previously stated, the term heated milk is a name applied to milk which has been pasteurized by the flash process. This type of pasteurization is gradually being replaced by the "holder process," as the latter has been found more satisfactory. The numbers therefore are smaller than those of the raw milk, or those of the milk pasteurized by the holder process. The results are grouped together in tables below.

HEATED IN BOTTLES.

Percentage of Age of Milk.

Months		Not Stated	Stated	24–36 Hours	36–48 Hours	48 Hours and over
January.....	100 samples	52	48	62	25	13
April.....	50 "	38	62	26	45	29
July.....	50 "	62	38	..	100	..
October.....	50 "	46	54	15	85	..

Percentage of Temperatures.

Months	Under 45° F.	45–50° F.	50–55° F.	55–60° F.	60–70° F.
January.....	77	21	2	..	..
April.....	52	14	22	12	..
July.....	12	50	32	6	..
October.....	66	24	18	..	..

PERCENTAGE OF BACTERIOLOGICAL COUNTS.

Months	Less than 10,000 Bacteria per c. c.	10,000 to 50,000 Bacteria per c. c.	50,000 to 100,000 Bacteria per c. c.	100,000 to 1,000,000 Bacteria per c. c.	1,000,000 and over Bacteria per c. c.
January.....	16	29	27	23	5
April:.....	10	32	16	40	2
July.....	18	50	14	18	..
October.....	20	36	16	28	..

The conditions shown in this table give fairly accurately the irregular results of the flash method of pasteurization.

Heated milk in cans.—As before stated, the milk known as heated milk is milk which has been pasteurized by the flash process. This process was gradually replaced during the year by the holding process, and we have, therefore, only 100 samples for the entire year. The results obtained are shown in Table 6.

Summary.—The conclusions based upon the preceding analysis may be summarized as follows:

1. The greater portion of the milk is delivered by the dairymen to the creameries with a low bacterial content, namely, less than 50,000 bacteria per c. c. Out of 20,344 samples, 14,389 were below the figure given.

2. The greater portion of milk sold in New York City as raw milk, whether in cans or bottles, contains from 50,000 to 1,000,000 bacteria per c. c. and a considerable percentage contains over 1,000,000 bacteria per c. c.

3. The pasteurization of milk by the holding process, though still leaving much to be desired, reduces greatly the number of bacteria, besides destroying any pathogenic varieties.

4. Ice is not sufficiently used to cool the milk.

5. The average temperatures of the milk as delivered to the creameries by the dairymen, with the exception of the winter months, is still too high for milk to be shipped to New York, and has an unfavorable influence upon the milk by aiding the growth of bacteria.

6. Greater care should be observed to obtain clean milk by sterilizing the cans and bottles.

7. The greater part of the milk is sold from 36 to 48 hours old, but a considerable portion is from 72 to 96 hours old, and some even more than 96 hours. This is true of both raw and pasteurized milk.

In closing, thanks are due to the former and present members of the bacteriological squad for the hardships undergone and the careful interest displayed in their work. Thanks are also due to the Misses Hazel and Helen Connors, and Messrs. J. J. O'Brien, E. J. McCullen, and T. Schroeder, for their share in the compilation of the data required for this study.

FATTY COMPOUNDS AS A FACTOR IN THE ETIOLOGY OF APPENDICITIS

Bertha Van Houten Anthony, M. D.

The following work was suggested after bacterial examination with both aërobic and anaërobic cultures† of the contents of 10 appendices had resulted in very irregular findings. This irregularity corresponds with the results of all other investigators who have attempted to place the cause of appendicitis on a bacteriological foundation alone. The following review of the work of Owen T. Williams suggests a most interesting problem. The attempts to verify his theory are given further on.

In the Bio-Chemical Journal of October 31, 1908, Owen T. Williams discussed the micro-chemical changes occurring in appendicitis. Clinical and chemical observations tend to show that there are conditions in the intestines in which certain soaps or other insoluble compounds of fatty acids are not absorbed by the intestinal mucous membrane and that with the presence of these certain diseases of the alimentary tract are related—as true intestinal sand, appendix concretions, etc. He had shown previously in an article on abnormal fat assimilation (British Medical Journal, July 27, 1907) that intestinal sand and appendix concretions are allied in chemical composition and are made up of compounds of saturated fatty acids (palmitic and stearic). Calcium and fatty acids are normal constituents of excretion of intestinal mucous membrane and it would seem that excess in secretion or defect in absorption might be the origin of true intestinal sand and appendix concretions. Thus the chemical constitution of these bodies may throw much light on the etiology of appendicitis, mucous colitis and similar processes.

In a large number of appendix concretions that he examined all showed concentric lamination and could be cut without difficulty. They are to be distinguished from masses of inspissated fæces, inorganic salts, gall-stones, false intestinal sands, and remains of undigested food which have no relation to the pathological processes accompanying fat digestion. He found true fæces in only three out of one hundred adult appendices at post-mortem. In the remainder occurred a dark slimy mucoid material similar to fæces in consistency, but not in pigment nor in chemical make-up. In one appendix were small granules which showed a likeness to true intestinal sand both in composition and in formation.

Although concretions occur by no means in all cases of appendicitis, yet Owen T. Williams shows that the process by which they are formed may go on in the wall of the appendix and give rise to morbid conditions which allow of easy invasion by micro-organisms, and thus lead to acute and chronic forms of appendicitis. These changes are the formation of calcium soaps in the sub-mucosa and mucosa of the appendix. Normally these soaps are expelled into the lumen and then into the cæcum, but in appendicitis they block the lumen of the gland or form a ring in the sub-mucosa. The micro-chemical changes in the walls of the appendix are demonstrated by certain staining reactions of:

Osmic acid which blackens neutral fats and not palmitic and stearic acids; Soudan III or Scharlach R. which stain neutral fats bright pink, soaps a pinkish yellow; nile blue sulphate, neutral fats pink, fatty acids violet; silver nitrate impregnates calcium compounds; petroleum ether dissolves the neutral fats, but not the soaps from a specimen.

Owen T. Williams found that appendices so stained show a large increase in the production of calcium soaps in the mucous membrane and sub-mucosa, observable also in sections stained in bulk. In some almost a complete ring is visible in the sub-mucosa. "Marked thickening," a change frequently recorded of the sub-mucosa, is due to the laying down

† The cultural work was done under the direction of Dr. Anna W. Williams in 1908, with material obtained from the New York Infirmary for Women and Children.

of these soaps. Small amounts of fatty acids are also demonstrated by nile blue sulphate, and calcium is found in definite amounts in the sub-mucosa of diseased appendices. A few show the excretion of these soaps into the lumen. Chemically, the excretions and the concretions show the same reaction.

Zuckerland (Nothnaegel—Diseases of Intestines and Peritoneum, 1905, p. 382) states that, as a result of age, with the atrophy of the mucous membrane and gland structures, the sub-mucosa undergoes thickening and the fat accumulates at that point. Yet in appendicitis the change in fatty compounds far exceeds (as Owen T. Williams has observed) any result of further involution changes. The formation of calcium soaps is a very slow process and must begin long before the acute attack of the disease, but by producing altered conditions of the mucous membrane or by acting as a barrier in the sub-mucosa and thus cutting off the nutritive supply, it renders easier the invasion by micro-organisms.

To test the above reactions a set of 48 appendices was stained. They were obtained chiefly from the New York Infirmary for Women and Children (four cases came from Bellevue Hospital morgue). The material was partly selected and falls in four groups:

(a) Acute appendicitis ..... 10 cases
(b) Chronic appendicitis ..... 7 "
(c) Chronic appendicitis with involvement of other organs ..... 18 "
(d) Autopsy appendices (seven from infants)... 13 "

In class (c) the appendix was removed usually as a routine measure during operation for other causes. In only two of these cases was a diagnosis of chronic appendicitis recorded before operation.

Four of the autopsy cases, class (d), serve as controls, as they showed no sign of intestinal lesions of any kind on examination at the morgue.

Method.—Since the alcohols dissolve out fats, the material after fixation in 10 per cent. formalin for one or more days was cut on the freezing microtome in the Pathological Laboratory of the New York Infirmary for Women and Children. The sections were rather thick, usually fifteen to twenty microns, and could be handled with ease. They were left in the stain about two or three minutes, washed in water and mounted in glycerine. Each section was treated with but one stain, yet as the pieces came from one small area of an appendix, the different pictures were practically comparable.

As an additional control, human fat from the mesentery of a case with no intestinal lesions (Bellevue Hospital) was stained in smears made both from the fresh material and from a portion kept in formalin twenty-four hours. There was no difference in the staining of the two lots. A part of the fresh fat was broken up into fatty acids; calcium soap was also made from it. This work was very kindly done for me by Mr. J. P. Atkinson.

CONTROLS FROM HUMAN FAT.

Stain	Fatty Acids	Calcium Soaps	Human Fat (Untreated)
Scharlach R.	Bright pink.	Dirty pink or yellowish pink.	Bright pink.
Soudan III.	Yellowish pink.	Pinkish yellow.	Orange.
Nile blue sulphate.	Blue.	Blue.	Rose pink (occasional purple tinge).
Osmic acid.	Black or gray.	Black or gray.	Black.
Silver nitrate.	Brown.	Grayish.	

From the beginning there has been a good deal of difficulty in interpreting the colors obtained with the above stains when applied to appendix material. Fatty acids are undoubtedly present to a greater or less degree in all cases except those of the infants. The presence of calcium soaps has not been so easy to determine, for to distinguish between a pink and a pinkish yellow (or a yellowish pink) is not always possible with the Scharlach R. or Soudan III. stains. Besides in the controls the nile blue sulphate gives both fatty acids and calcium soaps a blue color. The violet shade described by Owen T. Williams is due doubtless to an admixture of the pink of the neutral fats present in varying amounts in many cases. Osmic acid was of very little value, as it was found to stain fatty acids, calcium soaps and unsaturated neutral fats indiscriminately. Petroleum ether, in my hands, did not dissolve out the neutral fats even though used with thin sections for an hour or more. The silver nitrate, on which so much depends for detection of calcium soaps, was also most unsatisfactory. In some cases calcium compounds were doubtless blackened by it, but usually not to the extent one would be led to suppose after observation of the Scharlach R. or Soudan III. slides. It may be that the presence of other soaps gave an exaggerated picture with these stains.

The normal presence of fat in the sub-mucosa of the appendix is given in a number of histologies and anatomies. Adami, Delafield and Prudden, Bailey, Stoehr (Lewis' edition) show it in the pictures, but make no mention of it. Ferguson, in the description of a cut, gives "many fat cells." Woodhead mentions fat cells and their stains, while Gray's Anatomy gives "at times adipose tissue." Neither Osler nor Cunningham makes any mention of fat occurring in the appendix. Zuckerland, as noted before by Owen T. Williams, says the laying down of fat in the appendix occurs with old age, coincident with atrophy of the mucous membrane. It is interesting to note that in nine cases of this test where very large amounts of fatty material were found, seven were from patients between thirty-two and fifty-one years of age; of the remaining, one was from a girl of fifteen, the other from an adult control case (exact age unknown). On the other hand, five of the seven appendices of infants, two days to two and a half years old, showed no signs of fat. One infant, four months old, had a slight trace, the other, two and a half years, showed a small amount of fatty compounds present. This last case had been diagnosed "acute suppurative appendicitis," microscopically. Of the nine cases in which a large amount of fatty material was found only one, that of the young girl, was acute appendicitis, the others were two chronic cases come for operation, five appendices removed during operation for other causes, and one from a control case (autopsy case at the morgue) where no intestinal lesions were noted.

While the results obtained by this method of staining correspond in some measure to those of Owen T. Williams, the difficulty in interpreting the colors is a drawback to a satisfactory determination of the presence or absence of calcium compounds. It may be said, however, that in this set of 48 cases the presence of fatty acids and soaps increases with the age of the patient, especially between 25 and 50 years, particularly in chronic cases. One marked exception to the general theory is that of Case 633, acute purulent appendicitis in a girl 19 years old, where the neutral fats predominate instead of fatty acids or soaps. Nevertheless, further work on micro-chemical lines may give even more promise of discovering the real cause of appendicitis and allied diseases. The field is one of the widest, involving not only histological, pathological, and bacteriological changes, but the most variable question of diet. To review Owen T. Williams a little further:

The nature of fats in food determines to some extent the nature of fats in the tissues. It would seem, therefore, that as these abnormal processes are set up by saturated fatty compounds of palmitic and stearic acid (not by the unsaturated compounds of oleic acid) the nature of fat in the diet might have some influence in their production, since the

Acute Appendicitis. Ten Cases.

(The Use of the Symbols Plus and Minus is an Attempt to Indicate the Amount of Material Taking the Selective Stains for Fats and Their Compounds)

KEY

| indicates trace
± indicates present
+ indicates moderate amount
++ indicates marked amount
+++ indicates more marked
++++ indicates very marked

Case	Age (Years)	Scharlach R.	Nile Blue Sulphate	Osmic Acid	Silver Nitrate	Interpretation of Stain
476	11	Yellow pink ±	Blue +	Black |	?	Calcium soaps Fatty acids
395	15	Yellow pink +++	Bluish violet +++	Black ++	0	Calcium soaps Fatty acids
468	15	Bright pink ++	Blue ++	Black ±	?	Fatty acids
591	15	Pink + Dirty pink |	Bluish violet +	Black ++	±	Calcium soaps (sl.) Fatty acids
382	18	Pinkish yellow ±	Blue +	Black ±	?	Calcium soaps Fatty acids

ACUTE APPENDICITIS. TEN CASES. (Continued.)

Case	Age (Years)	Scharlach R.	Nile Blue Sulphate	Osmic Acid	Silver Nitrate	Interpretation of Stain
633	19	Bright pink ±	Pink + Violet |	Black ±	?	Calcium soaps and Fatty acids (sl.) Neutral fats
613	22	Bright pink ± Dirty pink |	Pink | Violet ±	Black ±	±	Calcium soaps (sl.) Fatty acids Neutral fats
620	29	Bright pink ± Dirty pink |	Pink ± Violet (sl.) |	Black +	|	Calcium soaps (sl.) Fatty acids (sl.) Neutral fats
592	33	Pink ± Yellow pink |	Purple ± Blue +	Black ++	|	Calcium soaps (sl.) Fatty acids
454	42	Yellow pink ±	Violet ± Blue ± Pink |	Black ±	|	Calcium soaps Fatty acids Neutral fats (tr.)

Case	Age Years	Scharlach R.	Nile Blue Sulphate	Omic Acid	Silver Nitrate	Interpretation of Stain
488	25	Yellow pink +	Violet + Blue | Pink |	Black +	?	Calcium soaps (sl.) Fatty acids Neutral fats (sl.)
478	27	Yellow pink ± Bright pink ±	Blue ± Violet ±	Black ±	?	Calcium soaps Fatty acids
405	29	Yellow pink ±	Violet +	Black ±	+	Calcium soaps Fatty acids
363	36	Yellow pink +++	Violet +++	Black +	±	Calcium soaps Fatty acids
390	37	Yellow pink +	Bluish violet +	Black +	?	Calcium soaps Fatty acids
470	41	Yellow pink ±	Violet ± Pink |	Black ±	o	Calcium soaps Fatty acids Neutral fats (tr.)
439	44	Bright pink +++	Violet +++	Black +++	?	Fatty acids

CHRONIC APPENDICITIS WITH INVOLVEMENT OF OTHER ABDOMINAL ORGANS. EIGHTEEN CASES.

Case	Age (Years)	Scharlach R	Nile Blue Sulphate	Osmic Acid	Silver Nitrate	Interpretation of Stain
397	24	Yellow pink |	Violet |	Black |	|	Calcium soaps (sl.) Fatty acids
475	24	Yellow pink ++	Blue ± Purple violet ±	Black ±	|	Calcium soaps Fatty acids Neutral fats (sl.)
403	26	Bright pink ±	Blue ±	Black +	+	Calcium soaps (sl.) Fatty acids
404	26	Bright pink |	Blue |	Black |	?	Calcium soaps ? Fatty acids (sl.)
453	28	Bright pink ±	Pink ± Violet ±	Black ±	o	Fatty acids Neutral fats
398	30	Bright pink |	Violet | Blue |	Black o	?	Fatty acids (sl.)
389	About 30	Bright pink +	Bluish violet ±	Black |	?	Fatty acids
385	32	Bright pink +++	Blue +++	Black +++	o	Fatty acids
513	35	Yellow pink ++	Purple blue ++ Pink ±	Black ++	?	Calcium soaps Fatty acids Neutral fats (sl.)

CHRONIC APPENDICITIS WITH INVOLVEMENT OF OTHER ABDOMINAL ORGANS. EIGHTEEN CASES. (Continued.)

Case	Age	Scharlach R	Nile Blue Sulphate	Osmic Acid	Silver Nitrate	Interpretation of of Stains
420	37	Bright pink ±	Blue +	Black +	o	Fatty acids
388	40	Bright pink ±	Blue +	Black |	o	Fatty acids
371	40	Yellow pink +++	Violet +++	Black ++	+	Calcium soaps Fatty acids
377	40	Yellow pink +	Blue ±	Black +	?	Calcium soaps Fatty acids
457	41	Yellow pink ++++	Bluish violet ++++	Black +++	o	Calcium soaps Fatty acids
399	43	Bright pink +++	Violet +++	Black +++	±	Calcium soaps (sl.) Fatty acids
409	43	Yellow pink ±	Violet + Pink |	Black |	±	Calcium soaps Fatty acids Neutral fats (sl.)
391	43	Yellow pink ±	Bluish violet ±	Black ±	?	Calcium soaps Fatty acids
406	51	Bright pink +++	Blue +++	Black +	?	Calcium soaps? Fatty acids

AUTOPSY MATERIAL. THIRTEEN CASES.

Case	Age	Scharlach R	Nile Blue Sulphate	Osmic Acid	Silver Nitrate	Interpretation of Stains
220A	2 days	o	o	o	o	No fat present
219A	4 months	o	o	o	o	No fat present
244A	4 months	o	o	Black |	?	Calcium soaps? Neutral fats (sl.)
221A	6 months	Yellow pink on m. m. tips	o	o	o	Calcium soaps (tr.)
228A	7 months	o	o	o	?	Calcium soaps?
245A	10 months	o	o	o	o	No fat present
227A	2½ years	Yellow pink |	Pink |	Gray on m. m. tips	o	Calcium soaps (sl.) Fatty acids (sl.) Neutral fats (sl.)
248A	37 years	Pink ±	Pink | Purple +	Black +	?	Calcium soaps? Fatty acids Neutral fats (sl.)
217A	70 years	Bright pink +	Violet + Purple ±	Black +	o	Fatty acids

AUTOPSY MATERIAL. THIRTEEN CASES. (Continued.)

Case	Age (Years)	Scharlach R.	Nile Blue Sulphate.	Osmic Acid	Silver Nitrate	Interpretation of Stain
*CA1	Adult	Bright pink +	Pink + Purple ± Violet ±	Black +	0	Neutral fats Fatty acids (sl.)
*CA2	Adult	Bright pink +++	Pink +++ Violet |	Black +++	0	Neutral fats Fatty acids?
*CA3	Adult	Bright pink ±	Pink ++ Violet ±	Black ++	0	Neutral fats Fatty acids (sl.)
*CA4	Adult	Bright pink |	Pink | Blue |	Black |	0	Neutral fats (sl.) Fatty acids (sl.)

*Controls.

CONDENSED TABLE

Diagnosis	Number of Cases	Fatty Acids			Calcium Soaps			Neutral Fats		
		+	Sl.	None	+	Sl.	None	+	Sl.	None
(a) Acute appendicitis	10	8	2	0	4	5	1	2	2	6
(b) Chronic appendicitis	7	7	0	0	5	1	1	0	2	5
(c) Chronic appendicitis (with other organs involved)	18	15	3	0	6	6	6	1	3	14
(d) Autopsy	13	2	5	6	0	5	8	3	4	6
(Including controls)	(4)	(0)	(3)	(1(?))	(0)	(0)	(0)	(3)	(1)	(0)

absorbability of the two is so different. A diet of olive oil, especially as used in salads, has been found beneficial in cases of intestinal lithiasis and mucous colitis, which seem so closely related to appendicitis, having in common abnormality of fat absorption. A further study of appendicitis in the meat-eating countries, as well as in those where more oils and vegetables are used in the diet, has been made. Statistics are necessarily incomplete for few countries have available data, but the marked variation in the incidence of appendicitis shows that it may be largely due to diet.

SUMMARY

One of the factors in the cause of appendicitis, as suggested by Owen T. Williams, may be the laying down of soaps and other insoluble fatty compounds in the mucous membrane and sub-mucosa of the appendix.

Conclusions.—That irregular cultural findings in various investigations (including our own) of the cause of appendicitis further this view.

That, as judged by the staining reactions of Scharlach R., nile blue sulphate, silver nitrate and osmic acid applied to a set of 48 appendices, fatty acids and calcium soaps predominate in the acute and chronic forms of appendicitis, while only neutral fats and slight amounts of fatty acids occur in the controls. In general these findings agree with those of Owen T. Williams.

That in this series the presence of calcium soaps and fatty acids seems to increase with the age of the patient, especially between 25 and 50 years.

That experience in the above work does not fully bear out the assumption of Owen T. Williams as to the ease with which calcium compounds are distinguished by means of these stains, since they bring in the error of correct interpretation of color.

That the incidence of appendicitis, as found by Owen T. Williams, varies in different countries with the diet, increasing with the use of meats and decreasing with the habitual use of vegetables and vegetable oils.

[Acknowledgment is due Dr. Katharine R. Collins, who called my attention to the article on which this work is based.]

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THE SO-CALLED "MUCH GRANULES" IN TUBERCULOSIS

Ira Van Giesen, M. D.

This question is synonymous with the contradiction of finding no tubercle bacilli under the microscope, and still proving their presence by the results of animal inoculation with companion portions of practically identical material.

This has been an old and puzzling problem to students of tuberculosis, and they have known for some thirty years that old, cheesy material, for instance, of bovine origin, especially, must contain bacilli in some form because it was virulent. But in spite of this, they were unable to demonstrate them under the microscope in smears or sections. The bacilli, or the remains of them, seemed absolutely invisible by the conventional staining methods of demonstration. The most reasonable explanation of the contradiction would be that such elusive bacilli had lost their acid-fastness, and then the puzzle would be simple, for such forms would be practically invisible with the methods current for their recognition.

According to his followers, who have built up quite a formidable literature, the solution of this important problem belongs to Much. This observer in comparing and contrasting the several methods of staining the tubercle bacillus found that the ordinary method of Gram not only appeared to stain more of the bacilli, but also stained here and there tiny granules and minute stavelets (Stäbchen) alongside of them. Having thus gotten the hint that the invisible tubercle bacilli might be non-acid-fast, and at the same time of unusual form in minute spherical or minature stave structures, he extended the intensity and scope of the Gram procedure by prolonged staining of hours instead of minutes.

Applying the modified method to material which was absolutely barren of bacilli by the conventional Ziehl method, it was no great task to convince himself and a good many others that these invisible and mythical bacilli had simply escaped detection by methods depending on the property of acid-fastness by losing this quality and had become transformed into these unfamiliar structures of isolated, apposed, or clustered granules and tiny stavelets.

Accompanying Much's work are experiments, which at first glance seem to grasp the question indisputably, although in reality they are extremely loose in catechizing the phenomenon and are wide open to sources of error. These experiments consisted quite naturally in planting bits of the seemingly barren tubercular material in culture media or in the tissues of animals, and from time to time with suitable staining technic watching the proliferation of the non-acid-fast miniature forms of the bacillus and their transformation into the familiar and classical structure of the bacillus in its acid-fast form.

Through abundant corroboration of Much's work, the problem now appeared to be settled. And to epitomize the whole matter the bacillus is regarded as having under certain circumstances a new life cycle. This, roughly speaking, comprises about one-third of its life history, which appears in a non-acid-fast form eluding detection by our time-honored methods.

One indication alone of the value of this "discovery" is that this masked part of the life history of the bacillus is coincident with phenomena of latent tuberculosis, and a revival of the theory of the transmission of the bacillus in this form from parent to offspring is at hand.

If the teaching of the Much school is true, its importance is secondary only to the discovery of the tubercle bacillus itself. But I feel that a good deal of this handiwork of Much's and his inductions are fallacious. And, far from solving the problem, he had not even properly approached it. Much, it appeared from the speaker's investigation on the subject, had fallen into one of those cleverly masked traps which beset every turn of

the scientific investigator. The pitfall in this problem seems to have been the conventional assumption that cheesy material and the like in tubercular lesions in dead stuff; it is structureless, amorphous, and contains nothing but the ashes of coagulation necrosis.

Consequenfly, when these tiny bits of chromatin in Much "granules" were disclosed in this supposedly lifeless material, it was natural to regard them as the only living constituent of this material, images of tubercle bacilli. In other words, these chromatin particles were the copies of what tubercle bacilli might be expected to resemble on the supposition that they had disintegrated or had lost their acid-fastness, although not their vitality to culture, nor their virulence to susceptible animals.

The only trouble with this accommodating theory is that it fails to eliminate the mimicry of cell degenerations. Far from having this degradation of structure and being devoid of chromatin life, cheesy material, the site of the invisible tubercle bacillus, when studied with especially devised technic shows an amazing complexity of organic structure and a wealth of chromatin particles emerging from hosts of leucocytes in all stages of degeneration. It was truly remarkable to witness the persistence of these degenerating leucocytes with their hosts of extruded chromatin filaments, and myriads of "granules" and "Stäbchen" mimicing what might be expected of degenerating non-acid-fast tubercle bacilli.

Undoubtedly in many of his preparations among the structures described by Much are involuting or aberrant types of tubercle bacilli; but the critical point still remains: that he has not eliminated the mimicry of granules and strands from the surrounding dying or degenerating cells. Quite likely also in some of his preparations he saw no vestigeal bacilli at all, but only their counterfeiting particles.

I feel that Much has not guarded himself against the similar mistake of considering the several "bodies" in cancer, variola, scarlet fever, rabies, etc., as the real transmissive organism. These, I believe, have been shown to be degeneration products. As a net result of Much's work, it would seem as if cumbersome methods had been substituted for the simple one of Herrman, published twenty years ago, which showed the granules in the substance of the tubercle bacillus without confusing it with other structures.

"PROWAZEK BODIES" AS A DIAGNOSTIC CRITERION AND MEASURE OF INFECTIVITY IN TRACHOMA

Caroline R. Gurley and Josephine T. Chase

The investigation here reported upon was undertaken with two main objects: *First,* to determine whether microscopic diagnosis of trachoma on the basis of the presence or absence of the Prowazek bodies was practicable; and, *secondly,* to collect data at first hand as to the degree of infectivity of the disease as it exists in New York City, especially in school children. For these purposes the nature of these bodies need not come into consideration, but only their specificity. The infectivity portion of the work was, until some months ago, carried on upon a very limited scale, and will, therefore, be dealt with more fully in the general report from the whole group of workers studying the subject, to be published later.

The work has been done under the direction of Dr. A. W. Williams, Assistant Director of the Research Laboratory; and from Dr. William H. Park, Director, we have received, from time to time, valuable suggestions and criticism. We wish to express our indebtedness and cordial thanks to Dr. G. W. Stoner, in charge of the United States Immigrant Station at Ellis Island, and to Drs. McMullen, Parker, and Dunn, of the Hospital there, for opportunities afforded us of getting some of our most interesting material. Also, we have been materially aided by Dr. Sarah J. Baker, Chief of Division of Child Hygiene of the Department of Health, in connection with work done in the schools; by Dr. H. W. Wootton, then in charge of the Department's clinics and Hospital for Contagious Eye Diseases; by his assistants, Drs. Wiesner, Harris, and Hough; and by several colleagues of the Research Laboratory.

Before detailing our own results, a brief outline of the status of the trachoma question is advisable by way of preliminary.

Résumé of Literature

The first step toward a possible solution of the etiology of trachoma was when Hess and Römer, in 1905, succeeded in transmitting the disease to anthropoid apes; the second, when in 1907, Halberstaedter and Prowazek[18] found in human trachoma and in experimental trachoma in orang-outangs inclusions in the conjunctival cells. In the orang-outangs after about seven days redness, swelling, and slight secretion appeared. In smears, according to Halberstaedter and Prowazek, the epithelial cells showed, near the nucleus, dark-blue, irregular, non-homogeneous, small, round or oval inclusions, staining with Giemsa's eosin-azure, similarly to the nucleolus. These become gradually larger, with growth increasingly falling apart, the process beginning in the center. Then inside these inclusions red-staining, distinct, very fine granules appear, rapidly increase in number, coming to occupy the larger part of the protoplasm, and displace the blue-staining masses which are now demonstrable only as small islands among them, and which gradually disappear. Prowazek gave the name *Chlamydozoa* (from the blue-staining *mantle*) to the group of supposed organisms. And later in 1907 he[39] characterized trachoma as a disease of the epithelia, in which the cells are enlarged, the virus multiplying markedly in them, leading to their rupture and its escape into the secretion; it is in the cells that the disease takes a chronic course. Follicle-formation is secondary to epithelial proliferation and associated blood stasis. Prowazek claimed to have demonstrated the developmental stages of the virus. He held that in a series of diseases, including trachoma, the host-cell responds to invasion by the virus, by characteristic and specific reaction-products, usually clearly demonstrable by nuclear stains. To the causative micro-organisms, the *Chlamydozoa,* he could not assign a fully

determined systematic position, but they seem to have greater affinity with protozoa than with bacteria.

Practically simultaneously with Halberstaedter and Prowazek, Greef, Frösch, and Clausen[15] described inclusions in the epithelia of the conjunctiva and secretion (sometimes also in Leber's *Körperchen* cells) consisting of just visible granules, sparingly present and usually paired, surrounded by a clear zone. Attempts at culture failed. The authors regarded the granules as the excitants of trachoma. Also, in November, 1909, Greef[14] again stated that he considered the specificity of the granules established, and that they are certainly the excitant of trachoma.

In August, 1908, Bertarelli and Cecchetto[4] said that the forms figured by Prowazek in his chlamydozoa article[39] are very rare exceptions, and differ in men and in apes. In the latter forms occur which could be cell-changes, and certain forms considered by Prowazek to be plastin masses may more probably be such changes. Other forms (*e. g.*, those consisting of collections of granules and scattered granules) can, however, less probably be considered degenerations. The authors claimed to have transmitted trachoma to lower apes (*Maccacus*).

In January, 1909, Stargardt[43] reported negative findings in 140 cases of conjunctival affections, except two early cases of trachoma, both positive. He named the inclusions "Prowazek bodies." He considered them specific. Nevertheless, finding typical bodies in a case of bacteriologically negative blennorrhœa neonatorum, he said perhaps other excitants exist capable of evoking the same cell-changes. Presence of the bodies speaks for trachoma, he thinks, but absence speaks neither for nor against.

Early in 1900 Thierfelder (*vide* Werner's summary) reported his finding of the bodies in two cases of conjunctivitis sicca, and in one of vernal catarrh.

Herford[22] reported on over 100 control preparations. Groups similar to Prowazek's can sometimes be found, but in them the granules are not so regular. Herford induced trachoma in a baboon, and demonstrated the bodies from the thirteenth day to the end of five weeks. According to him, in the human the granules are usually demonstrable (sometimes only after long search) as long as secretion and glassy follicles remain. He considered the granules parasites.

At the International Medical Congress at Budapest, September, 1909, Heymann reported Prowazek bodies from cases presumably non-trachomatous. In 119 cases[25] bodies were found in nine—in five out of ten cases of fresh trachoma, and in four out of seven cases of blennorrhœa gonorrhoica (also six more cases of the latter examined later were all positive). He considered the bodies a cell reaction to the gonococcus.

Gutfreund[16] analyzed 106 cases of trachoma; 47 were positive. The duration as far as ascertainable was: First day, eight; second to third day, ten; fourth to seventh day, six; eighth to fourteenth day, six. One case was positive after nine weeks; one after more than a year; and one was positive that had been treated, but not for a long time before examination. Several positive cases were repeatedly positive (some after four weeks in spite of treatment) and negative cases often gave a positive result on subsequent examinations.

In October, 1909, Werner[45] reported on fresh, untreated, treated, and old, scarred trachoma, and more than 25 controls (normal and acutely and chronically inflamed conjunctivæ, follicular conjunctivitis, vernal catarrh). He distinguished at length between specific and non-specific granules. Of the Prowazek bodies the rarest forms are those with intact mantle, inclosing relatively few granules; cells containing two groups of granules are also rare.

The same month Halberstaedter and Prowazek[20] published investigations on fresh or only slightly treated urethral gonorrhœa in men, and 25 cervical and female urethral gonorrhœas, all with negative results; and three gonococcus-free blennorrhœa neonatorum cases, which showed many inclusions. They concluded that the bodies are independent of the gonorrhœal process.

In November, Lindner announced his successful inoculations with a macacus and a baboon (attempt at transmission to two of each was made) with vaginal secretion of a woman whose child had so-called inclusion blennorrhœa. In the *macacus*, after seven days a mild and after fourteen days a moderate conjunctivitis occurred, with slight swelling and secretion; and in three weeks small granulations were present in the lower transitional folds. Inclusions were absent. The baboon showed on the eleventh day a relatively marked conjunctivitis, with many inclusions. He concluded that the vagina contained a virus which induces in the new-born a conjunctival inflammation not distinguishable from blennorrhœa gonorrhoica, and which in apes induces a disease clinically, pathologically and histologically similar to true trachoma. And in a later paper[32] he said that with a new method of staining he had demonstrated the bodies in old and treated trachoma—one eight-year, two six-year, one five-year, two three-year, etc. The inclusion blennorrhœas form, he thinks, a separate section of the bacteriologically negative blennorrhœas of the new-born.

In regard to the "trachoma" of New York school children, Alger, in 1910,[2] insisted strongly that the large majority of the cases are the pure follicular type, not at all contagious, unattended with symptoms, running a benign course, and that operation is not advisable. Acute trachoma, on the contrary, is much more rare in school children, and when found should lead to isolation, it being on an altogether different plane as regards infectivity. Alger devotes considerable space to treatment.

In April, 1910, Halberstaedter and Prowazek[21] proposed to call the bacteriologically negative cases showing Prowazek bodies chlamydozoa blennorrhœas. In one case out of two cases examined they found typical inclusions in cells from the meatus urinarius of a woman whose child had gonococcus-free chlamydozoa-blennorrhœa. The findings, they think, speak as well for the parasitic nature as for the etiological significance of the chlamydozoa. And this receives support, they say, through the transmission experiments of Lindner, who, with Hofstaetter, inoculating apes with secretion of the child and vaginal secretion of the mother, found epithelial inclusions like those in the ape conjunctiva inoculated with trachoma virus. The authors consider the separation of chlamydozoa-blennorrhœa from gonococcus-blennorrhœa further justified by the non-transmissibility of the latter to the ape conjunctiva. Flemming obtained similar results in the transmission of chlamydozoa-containing blennorrhœal secretion to the ape conjunctiva; and it plainly appeared that only secretion from chlamydozoa-blennorrhœa gave rise to inclusions in the ape conjunctiva. Also, Lindner and Fritsch demonstrated typical inclusions in non-gonorrhœal urethritis in men, and found the inclusions again in the conjunctiva of inoculated apes. And Halberstaedter and Prowazek themselves in non-gonorrhœal urethritis in men found within the cells groups of characteristic granules; plastin masses were not seen. Lindner and Fritsch conclude, the authors remark, that occurrence of inclusions in the cells of the female genitalia and of the male urethra in non-gonorrhœal inflammation, together with transmission to apes from blennorrhœal secretion, completes the chain of proof that these cases are all evoked by the same virus. And since Lindner and Hofstaetter have induced typical trachoma in apes through inoculation of blennorrhœal chlamydozoa, Halberstaedter and Prowazek regard this experimental proof as decisive, and assent to Lindner's views (changing their former opinion). Also, they admit the probability of mixed infection with gonococci or other micro-organisms.

Heymann[26] examined normal and diseased conjunctivæ of 235 men and 25 animals (baboon, *Cercopithecus*, dog, guinea pig, mouse). Prowazek bodies occurred only in trachoma and in conjunctival affections of nurslings, except in one trachoma-impossible case in a puerpera. Of 15 very fresh, untreated or very slightly treated trachomas, 10 showed the bodies; of 15 fresh recurrences, three showed them; but 23 old cases were negative. Of 45 cases of conjunctivitis in nurslings, 14 were pure gonococcus cases, 10 bacteriologically negative inclusion blennorrhœas, and 12 mixed blennorrhœas. While very numerous gonococci (demonstrated by culture)

were found in the same field (the same cell, even) with Prowazek bodies, still a certain opposition seemed to exist between the severity of the gonorrhœal infection and the number of Prowazek bodies. The most important result is, in Heymann's opinion, that the bodies demonstrated in non-trachomatous conjunctivitis and in genital secretion cannot be, in spite of the often established coincidence of gonococci, reaction products of the gonorrhœal process, but rather authenticate the existence of another, unknown virus, capable of multiplication and transmission to apes.

In 1910 Herzog,[34] who considers his results in harmony with those of Heymann, published an article in which he maintained that trachoma is due to a gonococcus infection occurring under certain conditions enumerated by him. He describes what he considers a hitherto unknown involution form of the gonococcus, the micro-gonococcus, which he claims to have cultured, and which he believes to be adapted to an epithelial-cell parasitism.

In January, 1911, Leber and Prowazek[30] reported on 114 cases of what they consider a new conjunctival affection in the Samoan Islands, 62 cases of which showed inclusions similar to those of trachoma. These they regard as chlamydozoa. In two of the cases trachoma bodies were also found.

In the same month Noguchi and Cohen[35] reported their studies of trachoma in New York City. No Prowazek bodies were found in smears from the cervix and urethra of three mothers of children with blennorrhœa neonatorum non-gonorrhoica. Of 60 trachomas with hypertrophy or pannus, 36 showed the bodies, as did also all of 14 cases observed from almost the beginning. In 11 cases of trachoma in children, with pannus or cicatrization, 7 were positive.

The bodies were absent from 15 cases of gonococcus blennorrhœa at Randall's Island. But three months later, in the same pavilion, of 30 cases in girls from 5 to 13, clinically like blennorrhœa gonorrhoica in adults, both tracoma bodies and gonococci were present, but with noteworthy inconstancy of association, sometimes bodies alone, sometimes gonococci alone, being present in smears taken every few days for four months. The authors concluded: The bodies are not due to local irritation; they are rarely associated with follicular catarrh (9 times in 250 cases), and their etiological significance is uncertain, their inconstancy of association suggesting that they are etiological for a conjunctival affection independent of, but clinically resembling, for a certain period, trachoma and also the papillary stage of blennorrhœa gonorrhoica.

Our Own Work.

After some preliminary studies had been made (the results of which are summarized in the appendix, for reasons given below) the broadening of the field of the investigation required an increase in the number of workers and the establishment of a separate clinic for especial observation of carefully selected cases. The clinic was started early in December, 1910, and was conducted by Dr. H. W. Wootton, Chief Ophthalmologist of the Department of Health. Patients have been sent from the three clinics maintained by the Department for contagious eye diseases, the larger number coming from Gouverneur Clinic, and hence from that portion of Manhattan below 14th Street and east of Third Avenue and the Bowery. The physicians in charge of those clinics were requested to send the cases considered by them to have trachoma or possible trachoma.

In addition, some microscopic examinations were made of material gotten at the United States Immigrant Station, on Ellis Island.

The cases selected for observation were almost exclusively either follicular catarrh, trachoma, or border-line cases. Of these, the first 224 are dealt with preliminarily in this report. The clinical diagnoses were all made by Dr. Wootton, instead of by one of several physicians as formerly, and a large number of cases similar to those which had before been diagnosed as granular trachoma were henceforth classed as follicular catarrh.

At our clinic the following routine is observed: If, upon diagnosis, the case is considered suitable for study, the history is taken; usually it is necessary to supplement the data on the occasion of the first visit to the patient's home, when, also, all members of the household present are examined and a note made as to the condition of each. In practically all the cases selected at least two smears of the conjunctival epithelium are taken, in order to examine for the so-called trachoma bodies. In case the child is not in attendance at another clinic, treatment is also initiated. Directions are, of course, given as to use of separate towels, pillow cases, etc., and mothers are instructed to have the child sleep alone if possible.

Technic.—The scraping of the conjunctiva in our earliest cases was done with the narrow end of a slide, but an ophthalmological sharp spoon (Knapp's Chalzion Curette No. 1) was found more convenient. When necessary, 4 per cent. cocaine solution was used.

The material dislodged was transferred to a slide and smeared out by laying a second slide upon the one bearing the scraping, and then drawing the upper one lengthwise over the other. Only such pressure as was necessary to spread out the clump was used, but this was usually considerable. The manner used in smearing blood was tried, but the fragments of mucous membrane scraped away are too tenacious to be spread out well in this way.

Fixation was done immediately for from 10 seconds to a minute in either absolute alcohol, absolute alcohol and ether (equal volumes), or methyl alcohol (Merck C. P.). The last two are, we think, to be preferred. Between these two the difference is slight. Some slides were rinsed in water immediately after fixation; other not, without appreciable difference in result.

Staining was always done with Giemsa's method; also, in addition, in some cases with Heidenhain's iron-hæmatoxylin method. Three solutions are kept on hand, preferably in the ice-box:

a.	French eosin (Grübler)	1%
b.	Azur I	1:1,000
c.	Azur II	0.8:1,000

At the time of using the eosin is first diluted by adding 2½ c. c. of it to 500 c. c. of distilled water. Twelve parts of the diluted eosin solution are then mixed with three parts of each of the azur solutions, and the mixture is filtered. Staining is done at room temperature from five hours to overnight or longer, or at 38° C. for from three to six hours. Sometimes the stain was used somewhat weaker, as follows: Seven parts of each azur solution were mixed with 40 c. c. of distilled water containing 0.15 c. c. of the 1 per cent. French eosin solution. After filtering, the staining was done overnight.

If Grübler's Giemsa solution is preferred, it is diluted for overnight staining in the proportion of one drop to one c. c. of distilled water, or even a greater dilution is used.

Prowazek bodies in smears.—What we designate as a Prowazek body consists of a varying sized group of very small, round or at times slightly oval, sharply contoured granules, lying within the cytoplasm of the epithelial cells, occasionally outside of the cells, and when so, in the immediate vicinity of an epithelial cell (or several cells) whose margin is not intact, and which contains one or more groups of similar granules. Stained with Giemsa, the granules vary somewhat in shade, being usually dark reddish-violet. Sometimes the granules forming the group lie in a clear space devoid of stained cytoplasm, and may be regularly disposed, but are not always so; at other times they appear within the cytoplasm, thickly crowded, forming a mass in which it is difficult to distinguish the separate granules except at the periphery; yet again they appear to be embedded in irregular masses of material staining in the shade of the nucleolus, the so-called plastin masses. In some cells the granules are very uniform in size; in others there is considerable variation.

These granule groups usually lie close against the nucleus, crescentic, with the concave surface fitting the nuclear rim, and not very frequently the same cell contains two groups, one on either side of the nucleus. At other times they lie at a distance from the nucleus in the cytoplasm. The groups vary widely in size. Occasionally a cell is entirely filled with granules, and in some instances shows a ruptured wall with granules outside, apparently escaped.

Noguchi states that: "There are two distinct varieties of granules in a moderately large heap, one minute and one coarse. The coarse granules usually lie along the periphery, while the finer granules are within the interior."

This statement, based upon smears, coincides with that of Wolfrum [47] based on section material, who says: "Two different zones of granules may be seen in the sections, a border zone which lies at the periphery of the cluster . . . and is distinguished by the extraordinary size of single granules, and a zone of inner granules, small and practically uniform. This characteristic arrangement is often not found in the upper layer of cells, but is frequently seen in the second layer. The number relation between the two zones of granules is regulated by the size of the clusters."

Nevertheless, though bearing this point in mind, we have been unable to detect any really definite layered arrangement. We, however, had no section material of any positive case. It is interesting to note in this connection the statement of Cecchetto [6] that the granules have always a uniform size.

As Bertarelli and Cecchetto have stated, no absolute faith can be placed in the color of the granules within limits, as this varies somewhat even with careful staining. It is upon a certain regularity of form—not, however, equality of size—and upon the manner of grouping of the granules that emphasis should be placed.

Even among definite groups a rather wide variation exists. Thus an occasional group consists of relatively finer granules than is usual, these being, as far as their crowded condition allows of judging, of about uniform size.

Other inclusions.—The variety of cell inclusions now and then met with is wide, and an interpretation often hazardous. Several authors have drawn attention to secondary cell changes which probably account for many of them. We have, however, observed the apparent inclusion within epithelial cells of a polynuclear leucocyte. This has also been noted in one case by Pascheff,[37] who figures also many other interesting inclusions, a number of which we have observed.

In a number of our control and trachoma cases were found the granules, certainly non-specific, mentioned by Bertarelli and Cecchetto [4] and described at length by Werner.[45] These are very fine, bright-red to reddish-violet (occasionally blue) granules, dispersed throughout the cytoplasm of the epithelium. Cells containing them often have nuclei and cytoplasm which stain normally, but more often the cells are clearly undergoing degenerative changes. In some cases only a very rare cell shows these; in other cases large numbers contain them. Again, in some cells they are of practically uniform size, while in others the size varies considerably; in number the variation is great.

Mast-Cells.—Parsons states that these are normal to the conjuctiva, and according to Herzog [23] they are abundantly present everywhere in the conjunctival epithelium. In our cases there was wide variation, a few showing quite numerous mast-cells, while the majority showed only rare ones. It may be that this is accounted for by the fact that some curettings were more superficial than others. Usually the coarse granules of these cells, being somewhat larger than the trachoma granules, and staining a deep red (Giemsa), cause no confusion; but occasionally a detached group may be misleading, since they do not invariably stain in sharp distinction to the more violet tone of the trachoma granules.

Frequency of Prowazek bodies.—Before attempting a classification of our "trachoma" material, it should be stated that no case has been called

positive unless at least one perfectly definite granule group (usually several or more such groups) was found.

Early in the work it became evident that conditions existed quite different from those met with during our preliminary work. Thus, cases showing Prowazek bodies proved disproportionately numerous. In part this was probably apparent only, and due to the fact that formerly, with very few exceptions, acute conjunctival affections were not examined for the bodies, because clinically they were regarded by the physicians in charge as non-trachomatous, or as observation cases.

In cases diagnosed as trachoma or border-line cases, which failed to show the bodies, repeated smears were very generally examined, with the result that not infrequently those which were negative on first examination proved positive later. Where the clinical diagnosis was "undoubted trachoma" and the bodies were not found the negative result, almost without exception, may, we think, be due either to the stage of the disease (old cases) or to the fact that the smears first taken were poor in cells and that it was not possible to secure additional ones.

In the nine miscellaneous cases where "trachoma" did not come into question and in the 66 cases diagnosed follicular conjunctivitis no bodies were found.

The following table summarizes the results obtained (total number reported upon, 224):

Original Diagnosis	No. of Cases	Cases Showing Trachoma Granules	Per cent. Showing Trachoma Granules
"Trachoma"	73	48	65.75
"Trachoma" of some standing	1	0	0
Doubtful	72	26	36.11
Probable trachoma	11	5	45.45
Possible trachoma	8	1	12.5
Trachoma or acute catarrhal conjunctivitis	16	7	43.75
Trachoma or follicular catarrh	36	13	36.11
Follicular catarrh	66	0	0
Other Diagnoses [1]	9	0	0
Undiagnosed [2]	4	4	100.

[1] Phlyctenular conjunctivitis, 3; subacute catarrhal conjunctivitis, 1; follicular conjunctivitis with superadded acute process, 4; trachoma, follicular type.

[2] Four undiagnosed cases which were negative are not included in the 224 reported upon.

Infectivity of Prowazek body cases.—Unfortunately our statistics are not yet abundant enough to admit of any definite statements regarding the degree of infectivity of the clinical group of conjunctivitis cases which corresponds to the presence of so-called trachoma bodies in the smeared epithelium. This is partly due to the fact that the present clinic was not started until early last December and that only a very small number of our "trachoma-granule" cases date back of January 10, 1911. Hence the families estimated upon have been observed less than five months, a period manifestly too short for drawing decisive conclusions.

The complete report of the group of workers studying "trachoma" will contain further results of this study.

It not infrequently happens that, when visiting the home of one of our patients in whom so-called trachoma bodies were found, we are told that one or more of the other children "had the same trouble" as the patient, but has been well for a longer or shorter period. That these statements may be correct is shown by Noguchi and Cohen's experience that in most of the cases of a series of 29 blennorrhœas in children studied by them showing the bodies (also gonococci) the conjuctiva resumed its normal appearance in three or four months; and by our own observation that in one family three children in whom the bodies were found had so completely recovered that when seen for the first time by Dr. Wootton about nine months after infection one was diagnosed as normal and two as having slight folliculosis. As determined by home visits, however, practically all symptoms had disappeared by the end of about three and a half months.

The three children belonged to a family of five, all born in New York, of Hungarian parents, who came to this country about twelve years ago. The father was dead. All three showed the Prowazek bodies, two in fair number, one in relatively large number. The mother and the other two children were warned of the danger of using the same towels, etc., but in spite of this the mother developed an acute inflammation clinically indistinguishable from that of the children. The smears in her case were negative for Prowazek bodies—it was only possible to take smear material once—but showed in fair numbers bacilli unlike those (exceedingly few) present in two of the affected children. The smears from the third child showed no bacteria. That the mother had trachoma is rendered very probable by the facts that her acute conjunctivitis outlasted the period of that condition when due to the other causes, and that, when seen some months later, it was found that the tarsal cartilages had been removed at the Manhattan Eye and Ear Hospital. In another family, also, of two children, smears from whom showed the bodies, one is, after about nine months, practically normal, while the other shows noticeable thickening and slight drooping of the upper lids. Precautions were observed, and the two other children, 14 years of age and over, have not become infected.

Then, too, in any attempt to estimate infectivity we must take into account the following two considerations: First, the fact that not infrequently members of the family suspected of having the disease cannot be gotten to the clinic for diagnosis, nor can smears be obtained; and, secondly, the very important point that careful directions are always given with a view to preventing the infection of others.

For these reasons the following estimate is to be regarded as suggestive only, and as probably representing a considerably lower degree of infectivity than really belongs to the disease.

The method of studying the question was, thus, by endeavoring to determine how many of the remaining members of a family, in which one member showed the Prowazek bodies, had contracted the disease, as evidenced by either (a) a diagnosis of undoubted "trachoma," by Dr. Wootton, or (b) the presence of the bodies in the smears. Thus, in this estimate we have assumed that other members of the family contracted the disease from the one first having it. The possibility remains, however, that in some instances the source of the subsequent infections may have been other than the home.

In 77 families tabulated, 323 individuals were examined, exclusive of the one in each who was taken to represent the source of infection; that is, an average of just above four in a family. Thirty-three out of the 323 were infected, or 10.2 per cent. It is interesting to note the smaller percentage of boys (9.2 per cent) than of girls (16.3 per cent). The percentage was very low in adults.

On the other hand, as far as our limited number of cases allows of judging, the statement of Alger is borne out, that follicular conjunctivitis is more frequent in boys, for out of 64 cases only 10 were girls; and in 24 of the 64 which were considered marked cases just two were girls.

CONCLUSIONS

1. In New York City "trachoma," as heretofore applied to school children, covers two principal conditions: (1) Folliculosis (when there are symptoms of inflammation it may be called follicular conjunctivitis), characterized generally by the absence of symptoms other than follicles and in the 66 cases covered by this report, by the uniform absence of the Prowazek bodies; and (2) papillary conjunctivitis ("inclusion-blennorrhœa" of Lindner), characterized by an initial stage with papillary hypertrophy, and acute inflammatory symptoms outlasting the period for acute catarrhal conjunctivitis, and by the presence of the so-called trachoma (Prowazek) bodies in the epithelial cells. This condition corresponds with that considered by most authors to be papillary trachoma.

As between these conditions, therefore, the absence or presence of the bodies will be found to be a valid diagnostic criterion. This criterion is also often valuable in post-operative cases in which diagnosis is obscured because of lesions which may be the result of the operation.

2. So far, no case of papillary conjunctivitis has been under observation from the acute onset until the advent of cicatrization or pannus, but the longest period of observation has been five months (with the exception of five cases) and during this time treatment has been carried out. We have, however, in three of the five cases constituting the exception seen restoration of the conjunctiva to practically normal after about four months.

3. Cases in which the "Prowazek bodies" are present seem in certain instances to have a rather high degree of infectivity. With such cases, therefore, special precautions should be taken to prevent the infection of others.

4. The advisability of microscopic examination for the bodies is suggested in (1) acute conjunctival inflammations outlasting the period for acute catarrhal conjunctivitis; (2) cases diagnosed "trachoma" having definite inflammatory symptoms; and (3) border-line cases (usually post-operative).

Appendix

The subject matter of the appendix represents some preliminary studies on a limited scale in which the diagnoses were made by several clinicians, and hence were felt to lack the unity involved in diagnosis by a single clinician.

The main value of this early work lies in the fact that its results are confirmatory of those subsequently obtained as regards the value of the so-called trachoma bodies in diagnosing between "trachoma" and follicular conjunctivitis.

All smears taken during the first three months of the investigation were carefully re-examined later, in order to rule out possible failure to find the bodies on account of initial inexperience.

CONTROL AND ELLIS ISLAND MATERIAL

As regards control material 13 cases were examined, 10 of which were acute catarrhal conjunctivitis, and three were normal conjunctivæ; all were negative.

Of the 24 cases of acute trachoma examined 17 were immigrants detained at Ellis Island, and with these, for convenience, is included one other, a male immigrant, aged 37, who came to this country several years ago. His case was of several months' standing at least, and he had been under somewhat irregular treatment with copper sulphate for four or five weeks. Prowazek bodies were present.

The Ellis Island material, like that from the last case, was collected for purposes of comparison, and was valuable as affording a class of cases clinically quite distinct from the large majority of the school children.

It was, unfortunately, impossible to obtain histories as to character of onset, symptoms, duration, etc., as none of the patients understood English, and in the few instances in which they spoke German they were from the South or East of Europe. The cases were 18 in number, ranging in age from 8 to 37 years, and included both sexes. Except in one case, only one examination was possible, because of the short time the patients are held at the Island. Of the 18, 16 were diagnosed as typical acute trachoma, one as fairly typical, and one was a very old case with extensive scarring, which was negative.

"Trachoma" of school children.—Among these children are included a small number below the school age, but belonging to families of which at least one child was in attendance at the clinic. It was, as a rule, impossible to get satisfactory histories. When questioned the children usually denied pain and discomfort, and said they only came to the clinic because the nurse told them to come.

As regards this very frequent absence of symptoms it should be remembered that in some of the cases there may have been previously a period of symptoms forgotten by the patient.

Apart from the control and Ellis Island material, 187 cases were examined, of which five were positive. All were at that time clinically diagnosed as trachoma, except four, which were called probably trachoma. The large majority of these cases were of the pure follicular type; in other words, those we now call follicular catarrh and folliculosis. Some were of mixed type, and very few were predominantly of the papillary type. Just two were acute, almost untreated trachoma, and one fairly early, which had been treated for several weeks.

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NOTES ON THE PRESENCE OF THE "NEGRI BODIES" IN THE RETINA IN RABIES

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Before taking up the question of the Negri bodies in the retina it seemed advisable to determine whether the retina contained the transmissive agent; in other words to test its virulence. This has undoubtedly been done, although we find no references to the question in the literature, except that of Frösch,[1] who states the retina is not infective. The quotation gives no details of these experiments, nor does it even state whether street or fixed virus was the subject of his experiments.

That the retina, however, should be virulent seems a foregone conclusion, since its structure in general does not depart radically from that of the general central nervous system, nor is it too remote for the virus to travel thither from the brain. One contingency, however, might arise in which the retina might fail to be virulent, namely, in the case in which a dog with ordinary street virus would be killed prematurely before the virus from the brain had time to reach the retina.

So, very likely there may be some capriciousness about the virulence of the retina in street virus, as is the case, for instance, with the cerebrospinal fluid, the pancreas, and the suprarenal capsules, peripheral nerves, aqueous humor, and mammary glands. Sometimes these structures are virulent and sometimes they are not. The retina probably falls in the class of organs not regularly or uniformly virulent. The sympathetic nervous system, the semilunar ganglion for instance, although it would be of considerable interest, does not seem to have been investigated very thoroughly as to the question of virulence.

The retina in fixed virus, one would suppose, is uniformly virulent. An emulsion of four retinas of two rabbits dead of fixed virus on the sixth day was inoculated subdurally into two new rabbits. These animals were paretic on the seventh and died of rabies on the ninth day.

Examination of the Retina in Fixed Virus.—Eight sets of retinæ were examined in rabbits dying of fixed virus inoculations on the sixth day. These were examined by smears and in sections. The smears are in general quite unsatisfactory. No indubitable bodies could be found, and it is exceedingly difficult, if not impossible, to recognize the fragile neuron bodies. And as these are few in number compared to the volume of the cells of the inner and outer nuclear layers, they become utterly lost amid the confusing elements of the retina strewn about on the slide.

In all of these smears there was a moderately abundant quantity of bright red shining hyaline disks (the smears were stained with a mixture of dilute fuchsin and methylene blue after drying in the air) with no chromatin particles. These spherules are quite uniform in size, about one-half a micron in diameter, and lie scattered about free and isolated. These resemble small Negri bodies without chromatin structure, well enough to pass at first glance as very good imitations of them, but we shall see later that there is good reason to believe that they are broken off segments of the rods.

The sections were taken from material fixed in various ways and stained in saturated eosin solution, followed by methylene blue. First, the globe was fixed in toto in 5 per cent. formalin, and the retina dissected out and embedded in paraffin. The material proved unsatisfactory both in fixation and staining reaction. Eyes dissected equatorially and fixed in Orth's fluid gave better results. Similarly prepared material fixed in Zenker's fluid gave the best results, but the retina became so brittle that it was found best in removing it from the globe to place it between two thin slices of hardened liver to keep it intact through the embedding process. The result of the examination of these sections is that Negri bodies are exceedingly scarce and difficult to find. For instance, in one

retina just two cells out of quite a large number examined in several sections contained inclusions of the achromatin type of Negri bodies; that is, each cell contained a single pale hyaline eosin-stained sphere about one or two microns in diameter, which was homogeneous and had no chromatin particles. Sections of the other retinas in fixed virus showed very much the same results. Some showed no bodies at all, while in others but one or two Negri bodies could be found in the entire section.

A further study of these sections developed the rather interesting feature of distinctive staining of the rods and tips of the cones. With fuchsin followed by methylene blue the rods and cones take the red dye brilliantly and retain it provided they are not passed through alcohol, but are mounted in lævulose or Brun's glucose medium. The rods are transversely segmented, and it appears that the slightest manipulation in technic is sufficient to detach bits of the cones, which then with their brilliant fuchsin stain give good imitations of achromatin-free Negri bodies.

Thus even in the most carefully prepared sections a few of these pseudo Negri bodies are found lying free among the tips of the rods, and occasionally some were found clinging to the surface of the limitans interna and indeed with some degree of proximity to the neuron bodies in the nerve cell layer. Undoubtedly this is the origin of the numerous pseudo Negri bodies in the smears of the retina mentioned above and also found in smears of perfectly normal and control retinas. In sections, of course, the possibility of displacing these detached fragments of the rods and shifting them over the neuron bodies is much slighter than in smears. And yet, if the retina were torn, or dissected out fresh and rolled up in a bundle for separate fixation, such a contingency is possible and might lead to mistakes.

Incidentally it was found that the rods and cone tips were partially acid-fast when stained with carbolfuchsin; thus they retained the dye with rosolic acid methylene blue decolorization, and to a less extent with 10 per cent. nitric acid. But the color did not remain fast to subsequent alcohol decolorization. It may be recalled that these structures also retain the dye in Weigert's hæmatoxylin stain as though their structure had in part something in common with that of myelin. And this community of structure with myelin may help to explain their partial acid-fast property. Negri bodies in air-dried smears are not in my experience acid-fast.

The Retina in Street Virus did not differ materially from that in fixed virus. Two retinas only from one dog (brain positive for Negri bodies) were examined. The bodies in sections are about one micron in diameter, are homogeneous and destitute of chromatin particles. Probably the retina in street virus is rather variable as to the presence of Negri bodies. Very likely a certain percentage of retinas from ordinary street rabies would show none at all, which would explain Frösch's statement, provided, of course, he was concerned with street virus rather than fixed virus rabies.

Retina in Human Rabies.—I have had an opportunity of examining the retina in a single case of human rabies. The smears of this retina in spots exhibits excellent views of the neuron bodies, probably because they are larger and seemingly less fragile than in rabbits or dogs. No bodies whatsoever were found either in the smears or in the Zenker's fluid-fixed sections. It is to be noted, however, that the Negri bodies were not particularly abundant in the brain of this case.

Control retinas from normal rabbits were also examined. They failed, of course, to show Negri bodies.

The only reference found on the subject of the occurrence of Negri bodies in the retina is the paper of Cornwall and Kesava Pai.[2] These authors state that they have found a further aid and a "quick way" for diagnosis in animals dying after fixed virus inoculations by the microscopical examination of the retina. To diagnose the nature of what we are perfectly certain of by experiment seems a gratuitous elaboration of

the obvious. However, these authors intimate that the Negri bodies in the retina are facile of detection and frequent in occurrence as compared to their presence in fixed virus brains. This is at such variance with my own results that I have been led to conjecture whether these authors may not possibly have been misled by these pseudo Negri bodies of this paper.

I am indebted to Dr. Williams, Assistant Director of the Research Laboratory, for suggesting and aiding this minor study.

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ANTIPERISTALSIS IN ITS RELATION TO TUBERCLE BACILLI AND OTHER BACTERIA IN THE ALIMENTARY TRACT

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The question of an antiperistaltic wave in the intestinal tract, of the existence of a muscular force which propels the intestinal contents in the direction of the stomach as well as toward the anus, was first investigated fifteen years ago by the physiologist Grützner.[1] He showed that if charcoal was injected into the rectum of an animal, for example a rat or a rabbit, particles of this substance could be found four to six hours later in the stomach. He also demonstrated that the same phenomenon was true for man, and for these tests used, besides charcoal, starch, sawdust, and other substances. This surprising conclusion immediately led other investigators to undertake similar experiments. In the next year we find experimental reports from Nothnagel's clinic[2] questioning the accuracy of Grützner's results, contending that if the animals were bound down so that they could not possibly aspirate particles of the materials used for the test, and if other errors were guarded against, none of the charcoal or other substances was found to have been carried to the stomach. Dauber[3] from v. Leube's clinic reached the same conclusions as the result of a series of seventeen experiments published the same year. On the other hand, almost simultaneously an investigation from Riegel's[4] laboratory confirmed Grützner's experiments both in animals and in man, and suggested as a corollary that nutritive enemata in man are absorbed not only from the rectum but also from the small intestine. In this country Hemmeter[5] reported in 1902 that he found in man that starch injected by rectum was demonstrable in the stomach seven to twelve hours later, and that following the injection of bismuth an antiperistaltic movement in the intestine could be traced by means of the X-ray.

It is difficult to come to a definite conclusion as the result of a review of these series of experiments conducted apparently in the same manner. It would seem, however, that occasionally particles of inert substances, such as charcoal or starch, may be found in the stomach after rectal injection. Interest in the question of antiperistalsis seemed to lapse for some years, but when next we find it the subject of investigation it concerns the antiperistaltic movement in its relation, not to inert substances, but to bacteria. The consideration of the movement of bacteria in the alimentary tract opened up an entirely new phase, as it introduced the subject of the infectious diseases, especially the mooted question of the portal of entry of the tubercle bacillus. This aspect of the question followed indirectly, but in logical sequence, v. Behring's dictum that the alimentary canal forms the chief route of infection in man for the tubercle bacillus. This connection came about in the following way. v. Behring's theory was soon supported by many other experimenters, notably by Calmette, Guerin, and their co-workers, who reported that if animals were given, by means of a stomach tube, material containing tubercle bacilli, these bacilli would in a short space of time pass through the intestinal wall, enter the thoracic duct, and find their way to the lungs, where they would later incite primary tuberculosis.

In answer to these experiments it was argued by those who believed in the acrogenic route of infection that in spite of the greatest care it was impossible to prevent the aspiration of tubercle bacilli when they were introduced through the stomach tube, and that probably the results of Calmette and others could be accounted for by the fact that some bacilli had gained access by way of the larynx and not by way of the intestine to the respiratory tract. In order to eliminate this serious and reasonable criticism, Schlossman and Engel,[6] performing a laparotomy, injected a suspension of tubercle bacilli directly into the stomachs of guinea pigs.

The experiments were performed with care; the incision in the stomach was closed by suture. After four to six hours tubercle bacilli were found in the lungs, thus substantiating v. Behring's and Calmette's theory as to the intestinal origin of pulmonary infections. It is not my purpose to discuss these results. I wish to mention these experiments merely in tracing the manner in which the question of antiperistalsis gradually came up for consideration in judging of the mode of infection of the tubercle bacillus. I may add, however, that Schlossman and Engel's results, although confirmed by some, were not substantiated in a series of experiments carried out later by Strassner,[7] who seared rather than sutured the gastric incisions, and in this way avoided the leakage which he believed to be incident to suturing. Ravenel and Reichel[8] carried out a similar series of experiments, but, owing to unfortunate circumstances, reached no definite conclusions; although in some instances the bacteria seemed to have traversed the intestinal wall and to have entered the lungs by means of the lymph stream. Rabinowitch and Oberwarth[9] reported positive results in experiments performed on young pigs which had been fed through a gastric fistula; previous to the feeding the esophagus was tied off.

In addition to the fact that the results of these various experimenters were not in accord, the objection was made that the severe operations to which the animals were subjected might essentially alter normal conditions, and that in many instances the dosage of tubercle bacilli was too large to furnish conclusions of practical value. However, all previous feeding experiments, those in which the bacteria had been implanted directly into the stomach, as well as the first experiments where they had been given the animals by means of gavage, were declared in 1906 by Uffenheimer[10] to be based upon a grave misconception of the physiology of the intestinal tract. He reported a series of experiments in which he had injected a suspension of bacteria per rectum into animals and found them within a few hours in the small intestine, stomach, esophagus, trachea, and lungs. From these results he concluded that there is a normal antiperistaltic movement from the rectum to the pharynx, such as Grützner described many years ago, which is able to carry bacteria along the wall of the intestine in a direction opposite to the normal downward current; that the bacteria, having reached the pharynx, easily gain access to the respiratory tract. An announcement of this nature was sure to excite notice and to lead to experiment, for it threatened to nullify all previous investigations relating to infections by way of the alimentary canal. The work of v. Behring, Calmette, Schlossman and Engel was all open to the criticism that the bacteria which they had considered to have passed through the walls of the intestine in reality had merely found their way to the lungs by way of the esophagus, pharynx, and trachea. Uffenheimer's work was repeated the following year by Bachrach and Stein,[11] who did not obtain the same results, but found that the bacteria introduced by rectum could not be recovered from the intestines above the ileo-cecal valve. However, although they suggested explanations for the contradictory nature of their results, they were unable to demonstrate where the discrepancy lay.

The most recent and the most comprehensive consideration of this subject appeared in 1908 by Dieterlen[12] in the "Studies on Tuberculosis" issued by the Kaiserliche Gesundheitsamt, which has been the source of so many excellent investigations upon tuberculosis. The conclusions of this author, whose article is entitled "Beitrag zur Frage der Infecktionswege," are: "Bacteria (prodigiosus, chicken cholera and tubercle bacilli), when given to rabbits by rectum, ascend in the intestinal canal in the opposite direction to peristalsis, through the stomach and esophagus to the pharynx, and are to be found regularly within one to four hours in the respiratory tract. If the ascent in the alimentary canal is obstructed by tying off the esophagus, the bacteria are generally not present within this short interval." "These results are of great importance in the consideration of the portals of entry of the tubercle bacillus."

After a careful scrutiny of Dieterlen's experimental data it seemed to me as if there was basis for a different interpretation from that which is quoted above, and which appeared so self-evident to the writer. On the contrary, it seemed impossible to coördinate his findings so that they should prove that the bacteria could have reached the respiratory tract only by way of the alimentary tract. Take for example the four experiments in which, previous to the rectal injection, he ligated the esophagus, in order to prevent the bacteria reaching the lungs by this route. Upon these experiments the author lays great stress, and yet upon analysis we find that two of them must be disregarded, for the bacillus prodigiosus did not reach the esophagus either below or above the ligature; moreover, in one of these instances it was unaccountably recovered from the urine. In the other two experiments, which were successful, as the bacillus prodigiosus was cultivated from below, but not from above the point of ligature, this organism was found also in the mesenteric glands in one case, and in the mesenteric glands, lungs, and the blood in the other experiment. Without going into detail it may well be asked—How is the migration of the bacteria to the lungs, after ligature of the esophagus, to be brought into harmony with their ascent through the alimentary tract? Three other experiments, in which the esophagus was not ligated, the author considers absolutely convincing ("ganz eindeutig"). In this opinion we cannot agree, for in one (No. 4) the bacillus prodigiosus was recovered only in the esophagus and left lung, whereas the lower portions of the gastro-intestinal tract, where we should also have expected to find the bacillus, were, so far as could be ascertained, absolutely free of them. In another one of these three experiments (No. 9) the same criticism holds, as the small intestine and stomach proved negative, whereas the esophagus gave positive cultures. In this case the blood of the heart showed the injected organism, and yet we note that the author in his discussion of the data strangely adds that examination of the heart's blood was always negative. Furthermore, the injected bacteria were found in several other experiments in the urine, once in the uterus, and once in the blood. But apart from these discrepancies it seemed hardly credible that bacteria could ascend from the rectum to the esophagus and lungs, a distance of about twenty-five feet, within the short space of an hour, as one of the experiments upon rabbits would seem to show. Taking into consideration these various factors, it appeared distinctly worth while, in view of the application of this subject to the route of invasion of the infectious diseases, to investigate further to see whether the data of previous workers could be corroborated, or whether if they could be accepted they might not be brought into harmony by subjecting them to another interpretation.

Original Experiments.—The technic followed, in general, was that used by Uffenheimer in his experiments. Eight-tenths per cent. salt solution was used as a vehicle for the bacterial suspensions. The enemata were given the animals through a sterile catheter, which was lubricated and then inserted to a distance of about two inches into the rectum. The fluid, at a temperature of about 98° F., was then gently and slowly injected into the bowel by means of a syringe. Following the injection, the anus was compressed for some minutes to prevent escape of the fluid; in almost all instances this was effectual. The animal was then slipped into a small bag which had been previously immersed in a warm bichloride of mercury solution. In order to prevent the animal from licking himself he was placed throughout the experiment, from the time he was prepared for inoculation until he was killed, in a box with an opening in the front large enough to admit his neck, but so arranged that he could not withdraw his head into the box. Thus the animal's body throughout the experiments was always within the box, whereas his head projected outside.

Most of the details of the experiments may be gleaned from a review of the tables which are given below. From these it will be seen that bacillus prodigiosus was used for the tests in the majority of cases. This

is the bacterium which has been employed in similar experiments on account of its distinctive color, which renders it easy of recognition, and because it is not to be found normally in the alimentary canal. The material for injection was obtained from forty-eight-hour potato slants, from which it was scraped by means of a platinum loop. Varying amounts of culture and of salt solution were injected. After an interval varying from seventy minutes to twenty-four hours from the time of the injection, the animal was killed, either by means of pithing through the medulla or by means of chloroform. The body then was flayed, washed with 5 per cent. carbolic solution, and stretched out upon a sterilized metal autopsy pan. The greatest care was exercised to avoid contamination of the organs during the autopsy, which generally was performed in a room different from that in which the injection had been carried out or the animal kept subsequent to the injection. That the bacillus prodigiosus was not present in the atmosphere of the autopsy room may be stated as the result of control which showed that of the many agar plates exposed to the air during the time of the autopsy none showed any of these micro-organisms. Each organ or part of organ was removed with separate sterilized instruments and placed in Petri dishes, the greatest care being exerted to avoid contamination with blood. The thoracic organs were first removed and then those of the abdominal cavity. In the case of the hollow viscera, the contents were obtained as follows: The trachea was excised with instruments heated in the flame and the contents washed into a Petri dish by means of salt solution. This same method was followed in the case of the esophagus, which was excised between ligatures; the ligatured ends were then removed by means of heated scissors, and the esophagus flushed out without being further opened. The stomach was removed in the same way, its surface seared, and contents expressed. Material from the wall was obtained after the large mass of contents in the center of the organ had been discarded. In some instances the animal had fasted overnight previous to the experiment, but in spite of this precaution food was found in the stomach. The duodenum, ileum, and parts of the large intestine were evacuated after the manner of the stomach. The bits of organs which were removed were thoroughly macerated and then transferred into large tubes containing thirty cubic centimeters of broth. Frequently three or four pieces of macerated tissue were placed in as many tubes, and thirty, forty, or even fifty tubes of broth inoculated for one experiment in order not to overlook any of the test bacteria. A similar method of culture was carried out with the contents of the hollow viscera. In the case of the small intestine, where the aërobic bacteria found were few in number, large amounts of the fluid contents were pipetted into the broth; from the stomach and large intestine smaller transplants were made. The broth tubes were then incubated at a temperature of about 26° C. for twenty-four or forty-eight hours, when numerous agar plates were made from them. Generally when the bacillus prodigiosus was present, after an interval of a week the broth showed a red ring about its upper border; in one instance this did not appear for three weeks; in other cases the organisms were so few that they could be identified only by means of plates.

Blood cultures were carried out in every instance. This consisted not merely of aspirating post-mortem a small quantity of blood from the heart, as has been done by previous workers in this field, but of obtaining as much blood as could be drawn from the ear vein or jugular vein, as much as fifteen, twenty, or even thirty cubic centimeters. This was caught in a solution of sodium citrate and then inoculated into numerous flasks of broth; plates were made from the flasks as in the case of the cultures of the organs. This difference of technic is, I believe, very important. In other experiments bacillus tuberculosis was injected. It would have been desirable to have used this organism for all experiments, but this was impracticable partly on account of the scarcity of guinea pigs. As the experimental data show, the various organs, the contents of the alimentary tract, the blood and the urine all had to be

tested by means of further inoculation into guinea pigs. Moreover, a disadvantage of using the bacillus tuberculosis was that many of the test guinea pigs succumbed to secondary infections, which detracted from the completeness of the experiment. In one instance I used a tubercle bacillus of the human type, in all others a culture of the bovine type which was virulent for rabbits, although it had been cultivated upon egg media for many generations. All guinea pigs were killed six or more weeks after inoculation.

Experimental Data

(*a.*) B. prodigiosus given per rectum to rabbits.

1. Weight of animal 1,500 grams. Inoculated with the growth of 9 potato cultures suspended in 19 c. c. normal salt solution. Killed by chloroform two and one-quarter hours later.
   Positive cultures: small intestine (1), large intestine (1), left kidney (1).
   Negative cultures: left lung (4), right lung (4), trachea (2), esophagus (2), liver (4), spleen (2), urine (2), blood from ear vein (4), heart (2), stomach (2).
   Remarks: other cultures from small intestines were sterile. Four c. c. of blood were obtained for culture after 70 minutes, four more just before animal was killed.
2. Weight of animal, 1,780 grams. Inoculated with growth of 19 potato cultures suspended in 18 c. c. of salt solution. Pithed after two hours.
   Positive cultures: sigmoid (2), colon (1), heart's blood (1), blood from ear vein (1).
   Negative cultures: left lung (2), right lung (3), upper trachea (1), lower trachea (1), upper (1) and lower esophagus (1), liver (2), spleen (1), kidney (1), stomach wall (1), and center (1), small intestine (3), urine (1), blood from ear veins (5), colon (1).
   Remarks: 3 c. c. of blood was obtained from ear vein previous to killing animal.
3. Weight of animal, 1,450 grams. Inoculated with growth of 10 potato cultures suspended in 16 c. c. salt solution. Pithed three hours later.
   Positive cultures: sigmoid (2), kidneys (2), liver (1), heart's blood (1), blood from jugular vein (1).
   Negative cultures: left lung (2), right lung (2), trachea (2), esophagus (2), spleen (1), blood from jugular vein (1), blood from ear vein (2), stomach (2), colon (2), liver (1), small intestine (2), heart's blood (1).
   Remarks: 2.5 c. c. of blood was used for the two cultures from the ear veins, about 8 c. c. for the two cultures from the jugular veins.
4. Weight of animal, 1,500 grams. Inoculated with growth of 12 potato cultures suspended in 18 c. c. salt solution. Bled to death after two hours.
   Positive cultures: blood from jugular vein (3), left kidney (1).
   Negative cultures: blood drawn from ear 50 minutes after injection, four cultures. Blood from ear two hours after injection, four cultures. Blood drawn from jugular vein two hours after injection, two cultures. Right kidney (2), heart's blood (2).
   Remarks: in this experiment 4 blood cultures were made from ear vein after 50 minutes and four at the end of two hours, at which time five cultures were made from the jugular vein.

5. Weight of animal, 1,500 grams. Inoculated with growth of five agar cultures suspended in 10 c. c. salt solution. Pithed after 24 hours.

Positive cultures: right lung (1), left lung (1), blood (1).

Negative cultures: sublingual gland (1), parotid gland (1), esophagus (2), trachea (2), stomach: (*a*) cardia (1), (*b*) pylorus (1), blood from jugular vein (3), duodenum (1), ileum (1), colon (1), urine (1), heart's blood (1).

Remarks: four blood cultures were made from jugular vein, using in all 12 c. c. of blood. One of these showed B. prodigiosus.

(*b*) B. prodigiosus injected intravenously into rabbits.

1. Weight of animal, 2,100 grams. Inoculated with growth of one 72-hour-old agar culture suspended in one c. c. salt solution. Chloroformed after two and one-half hours.

Positive cultures: esophagus (1), trachea (1), small intestine (1), sigmoid (2), bile (1).

Negative cultures: stomach (3), small intestine (1), colon (2), rectum (2), urine (1).

Remarks: the cultures from the esophagus and trachea were positive, due possibly to the cutting of a small blood vessel in their neighborhood in the course of their excision.

2. Weight of animal, 1,750 grams. Inoculated with growth of one 72-hour-old agar culture suspended in one c. c. salt solution. Chloroformed after one hour.

Positive cultures: small intestine (2), liver (1), bile (1).

Negative cultures: esophagus (1), trachea (1), stomach (3), small intestine (1), large intestine (4).

(*c*.) B. tuberculosis given by rectum to rabbits.

1. Weight of rabbit, 1,700 grams. Inoculated with 19 c. c. of a suspension of tubercle bacilli of the human type in salt solution, the solution having the density of a 24-hour typhoid culture. Secondary inoculations of organs into guinea pigs. Pithed after three and three-quarter hours.

Positive inoculations: none.

Negative inoculations: spleen, stomach, kidney, liver, colon (2), small intestine, heart's blood (1), 2 c. c. blood from ear.

Inoculations causing death by secondary infections: left lung, right lung, trachea, esophagus.

Remarks: spreads from small intestine and from colon failed to show tubercle bacilli. Cultures from lungs, trachea, liver, kidney, spleen, stomach, and small intestine did not show bacilli coli.

2. Weight of animal, 1,960 grams. Inoculated with 18 c. c. of a suspension of tubercle bacilli of the bovine type, of the same density as in Experiment No. 1. Secondary inoculations into guinea pigs. Bled to death after two and one-quarter hours.

Positive inoculations: none.

Negative inoculations: esophagus, stomach, urine, heart's blood (1), blood from jugular vein (6).

Remarks: the blood was caught in sodium citrate solution. Three pigs developed convulsions, followed by paralysis and death, as the result of intra-abdominal inoculation of blood. After allowing the citrated blood to stand in the ice-box overnight the guinea pigs withstood the inoculations. Two guinea pigs inoculated with the washings from the pelves of the kidneys showed the same phenomena.

3. Weight of rabbit, 1,600 grams. Inoculated with 45 mm. of bovine tubercle bacilli finely suspended in 6 c. c. salt solution. Bled to death from jugular vein after 24 hours.
   Positive inoculations: left lung (1), blood (2).
   Negative inoculations: left lung (1), right lung, blood (4).
   Remarks: 16 c. c. of blood was obtained in sodium citrate solution, allowed to stand overnight in the ice-box, and the sediment inoculated intra-abdominally into six guinea pigs. None died. Two developed tuberculosis.
4. Weight of rabbit, 1,530 grams. Duplicate experiment of No. 5, only that more organs were inoculated: 50 mm. of the same bovine culture were injected in 6 c. c. salt solution. Bled to death after 24 hours; about 30 c. c. of blood obtained.
   Positive inoculations: urine (1), blood (4), mesenteric glands (1), stomach (1).
   Negative inoculations: small intestine (2), esophagus (1), mesenteric glands (1), stomach (1), left lung (1), right lung (1), blood.
   Remarks: 17 guinea pigs were used in this experiment; one inoculated with macerated piece of left lung died of a complicating infection.

(*d.*) B. tuberculosis inoculated intravenously.

1. Weight of animal, 1,250 grams. One hundred and thirty milligrams of a bovine culture suspended in normal salt solution injected into ear vein. Pithed after three hours.
   Positive inoculations: blood (3), stomach (2), small intestine (2).
   Negative inoculations: small intestine (1).
   Remarks: in this experiment and others in which intravenous inoculation was employed the greatest care was exercised to avoid contaminations of the contents of the viscera by means of blood. The walls of the stomach and of the intestines were seared and then incised with a knife heated in the flame.

A review of the experimental data detailed above shows that after the bacillus prodigiosus had been given by rectum to rabbits it was rarely found in the alimentary tract above the ileo-cecal valve. In one instance it was recovered from the small intestine, but never from the stomach or from the esophagus. It was, however, found in the blood, in the kidneys, in the lungs, in the liver, and in the bile. It was recovered from the blood in all but one instance, namely, in the first experiment, in which the technic was not yet well developed. From these results it seemed clear that this organism when introduced per rectum was readily absorbed from the intestinal canal. It therefore seemed a logical deduction that the bacilli gained access to the kidneys, lungs, liver, and bile by way of the blood current. That they did not ascend to the lungs by way of the alimentary tract, as has been asserted by others, was evident, as the bacilli could not be recovered from the esophagus or stomach. It must be acknowledged that they might well have been overlooked in the latter organ owing to the large amount of food which the stomach of the rabbit contains even when the animal has not partaken of food for many hours, but it was not possible that this error could occur constantly in an examination of the esophagus, when we remember that our technic consisted of a bacteriological test of the complete washings of this organ. It may be that those who recovered the bacillus prodigiosus from the stomach and from the esophagus obtained this result at times because they did not consider it necessary to prevent contamination by way of the blood; if this danger is not carefully guarded against, the vessels upon the outer surface of the stomach or esophagus may bleed upon incision, and it might so happen that infected blood would mix with the contents of

these organs. However this may be, the presence of the bacillus in the blood current explains many of the factors in the data of the previous workers which seemed inconceivable when considered from the point of view of a distribution of bacteria solely by means of antiperistalsis. It explains the presence of the bacteria in the urine, in the lungs, in the trachea and in various other organs. In one of our experiments it will be noted that the bacillus was recovered only from the blood and the lungs; this would appear quite inexplicable if we must believe that the bacteria ascended to the lungs by way of the alimentary tract. That Dieterlen, in his recent investigation from the Kaiserliche Gesundheitsamt, and previous workers failed to find the organisms in the blood can readily be understood. This was due to the same error that formerly led to negative results in the examination of the blood in pneumonia and typhoid fever—the employment of an insufficient quantity of blood for cultures. The use of about two or three cubic centimeters of blood may well lead to negative results. In one experiment (2, *a*), where the blood was examined two hours after the rectal injection, five blood cultures proved negative, but the sixth one showed the bacillus prodigiosus; in another experiment (3, *a*) two cultures of one and one and one-half cubic centimeters of blood from the ear veins were sterile, a culture of four cubic centimeters of blood from the jugular vein was likewise sterile, whereas a second culture from this vein gave a positive result. If we had depended upon a single culture of a small amount of blood from one of the veins or upon the examination of a small quantity of blood aspirated from the heart (4, *a*), as has frequently been done by earlier workers, we might well have considered the blood sterile.

As I have mentioned above, the employment of the bacillus tuberculosis for these experiments is somewhat unsatisfactory because many of the guinea pigs made use of to show the presence of this organism in the various organs (intestines, lungs) succumb to secondary infections. In two experiments where the rabbits were killed a few hours after the rectal injections of tubercle bacilli, once of a suspension of bacilli of the human type, another time of bacilli of bovine type, the bacilli could not be demonstrated in the small intestine, stomach, or esophagus. However, they could very well have escaped detection in the examination of the intestinal and stomach contents, owing to the small amount that was used for inoculation, but had tubercle bacilli been present in the esophagus they would certainly have been found. In both of these experiments the organism was not found in the blood, which shows that the tubercle bacillus does not traverse the intestinal wall as readily as does the bacillus prodigiosus. In two other experiments (3, *c*, 4, *c*), where twenty-four hours were allowed to elapse following the rectal injection and previous to killing the animal, the tubercle bacillus was found in the blood. In one of these (4, *c*) it was demonstrated also in the mesenteric glands, which clearly indicated its route from the intestines to the blood stream. It should be noted that in this experiment tubercle bacilli were found in the stomach. There is no means of deciding whether their presence in this organ was due to antiperistalsis, or whether they gained access by way of the blood. It may, however, be mentioned in this connection that when tubercle bacilli are injected into the blood they are to be found in the stomach as well as in the small intestine within a few hours (Experiment 1, *d*). In similar experiments performed with B. prodigiosus it was found that after inoculation into the ear vein, although the liver and the bile showed the greatest number of bacilli, they also were present in the alimentary tract. It is therefore certainly possible, although we cannot judge of the probability, that in the experiment cited, where the bacillus tuberculosis was found in the stomach twenty-four hours after rectal injection, it reached this organ indirectly by way of the blood.

One clinical test was made. A "typhoid-carrier" who is known to have had typhoid bacilli in her stools for some years, at times almost in pure culture, but at the time we made the test in the ratio of about 10 per cent. of the total number of fæcal bacteria, was submitted to an

examination. It seemed that in a case of this nature, if bacteria are carried by means of antiperistalsis upward from the intestines, typhoid bacilli should be present in the stomach or even in the pharynx. In order to gain information on this point, the patient, after partaking of no food for eight hours, was asked to gargle with sterile salt solution, and this fluid was tested for typhoid bacilli. Her stomach, which was found to be empty, was next washed out, and the washings, neutral in reaction, tested by means of the Conradi-Drigalski culture medium. Neither the fluid from the pharynx nor that from the stomach was found to contain typhoid bacilli. Moreover, in this case the cultures also failed to show any colon bacilli, which we should expect to find in the stomach and pharynx if antiperistalsis played an important part in the physiology of man. This is merely a solitary experiment and should not be made the basis of sweeping conclusions; however, in this connection I would note that several tests were made on rabbits to demonstrate at autopsy the colon bacillus in the lungs, trachea, stomach, and small intestine, and they also gave negative results.

In view of the results obtained, we believe that when bacteria are injected into the rectum the antiperistaltic wave does not, as a rule, carry them above the ileo-cecal valve. The contrary conclusion, namely, that they rapidly ascend from the rectum, along the intestinal wall into the stomach, esophagus, pharynx, and thence into the respiratory tract, is based upon a mistaken interpretation of experimental results. The bacteria are certainly found in these organs following rectal inoculation, as has been claimed, but they gain access there, not by way of the alimentary tract, but by way of the blood. They traverse the intestinal wall, enter the lymphatic system and are then distributed by means of the blood stream throughout the body. This is true of tubercle bacilli as well as of the bacillus prodigiosus, the only difference being that the former was not found to traverse the intestinal wall and enter the blood so quickly The reason why previous workers have failed to find the bacilli in the blood, following rectal injection, is merely because they employed insufficient quantities of blood for their cultures or animal inoculations. Finally, in view of these conclusions, it does not seem that there is experimental basis for believing that antiperistalsis is an important factor in relation to the infectious diseases.

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THE ELIMINATION OF BACTERIA FROM THE BLOOD THROUGH THE WALL OF THE INTESTINE

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It has been recognized for many years that the wall of the gastro-intestinal tract forms one of the numerous paths of excretion of foreign substances which have entered the blood. One of the earliest proofs of this vital phenomenon consisted in the injection of antimony subcutaneously, and after a short interval the demonstration of the presence of this drug in the stomach. Various other drugs and poisons, such as morphine, atropine, strychnine and snake-venom, have similarly been proved to pass from the blood through the gastric or intestinal wall, following intravenous or subcutaneous inoculation. In fact this mode of excretion is so well established and so thoroughly accepted that it has led to the routine employment of repeated washing of the stomach and colon in the treatment of poisoning by these drugs. Various other chemical substances, for example, strontium,[1] barium,[2] lithium,[3] manganese[4] and bismuth,[5] have been shown to follow, to a greater or less extent, the same excretory route. All these experiments have one essential point in common, namely, that a soluble salt was used for the test, such as lithium citrate, strontium, acetate, or bismuth tartrate. No report has been made of similar tests carried out with insoluble salts.

The experiments reported in this paper do not consider the excretion of salts, but were undertaken to ascertain whether bacteria are excreted from the blood through the intestinal wall. These experiments were suggested by some previous work which showed the great ease and rapidity with which bacteria are absorbed by the intestinal mucosa. Since this work was undertaken I have found from a perusal of the literature that this problem is not entirely new, but was considered about twenty-five years ago in relation to the cholera bacillus. Emmerich[6] believed that he could recover his so-called cholera bacillus from the intestine some hours after injecting it into the blood or beneath the skin, and that the bacillus had traversed the intestinal wall. Buchner[7] supported him in this belief. Whatever may have been the facts, the experiments performed to demonstrate this theory were by no means adequate. No care was exercised to exclude the entry into the intestine of the bacilli by way of the bile duct; indeed, Buchner records that they were cultivated from the liver. So these experiments must be regarded merely as raising the question which forms the nucleus of this paper, but in no way as affording proof of the theorem which Emmerich and Buchner proposed. This is the most serious, but not the only, weak point in these early experiments, which it is unnecessary to dissect any further. It may be added that most of the experiments performed with the soluble salts are open to the same criticism. In this later work, however, other factors, such as the finding of the salt in the stomach or the local lesions of the mucous membranes, render the interpretation of Emmerich and Buchner in most cases justifiable.

In the course of some work carried out on the subject of antiperistalsis in its relation to bacteria,[8] which is the work above referred to, it was found that bacteria which are introduced into the rectum are rapidly

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[7] Buchner, H.: Beitraege zur Kenntniss der Neapeler Cholera Bacillen, etc., Arch. f. Hyg., 1885, iii, 361.

[8] Hess, A. F.: Antiperistalsis in its Relation to Tubercle Bacilli and other Bacteria in the Intestinal Tract, Jour. Med. Research, 1910, xxii, 129.

absorbed into the blood current; this may be demonstrated by means of blood cultures, provided sufficient blood be employed for the tests. The question next suggested itself as to whether the reverse is true, whether bacteria can readily be found in the intestine, if they are introduced directly into the blood stream. Accordingly some preliminary experiments, with this question in view, were undertaken; these were mentioned in the paper on antiperistalsis. It was found that in some instances in which tubercle bacilli were injected intravenously they could be recovered three hours later from the contents of the stomach and of the small intestine; that in another experiment in which the *Bacillus prodigiosus* was used as a test organism this bacterium could be regained from the small intestine one hour subsequently. In order to test the possibility of these bacteria having gained access to the intestine by way of the lungs and the upper respiratory tract, two further experiments were conducted. In these the pylorus was ligated preliminary to injecting the bacteria, in order to obstruct effectually this means of entrance into the intestinal tract. In both experiments the *Bacillus prodigiosus* was isolated from the contents of the small intestine. The subject was pursued no further at that time and the question of the path of entry of these bacilli into the lumen of the intestine was left undetermined. It was realized from the work of others that many of the bacilli no doubt were excreted by means of the bile and had reached the intestine by this route. It seemed possible, however, that some might have traversed the intestinal wall. No conclusive data on this point could be found, and accordingly experiments were begun some months later to determine this question.

EXPERIMENTS

As elimination by means of the bile of a substance injected into the blood could be presupposed, the first series of experiments necessitated ligation of the common bile duct. This duct was either doubly ligated, or, in addition, incised just where it enters the wall of the upper duodenum. The operation, performed on rabbits under ether narcosis, was conducted quickly under aseptic precautions, and entailed but slight handling of the intestine. The animals which were subjected to this or to subsequent operations were inoculated as soon as they had recovered from the narcosis; that is, after an interval of one or two hours. The test inoculation was performed after this short interval rather than after the lapse of a longer period, because it was considered that the longer the bile was dammed back after ligation of the bile duct the greater was the departure from physiological conditions. Of the three operations in which this procedure was adopted the *Bacillus prodigiosus* was recovered from the small intestine in two. In the first (Experiment 18) three platinum loops (4 mm. in diameter) of culture were injected and the bacterium was recovered two hours later. It was isolated from the duodenum and the upper ileum, but not from the lower ileum. In the second experiment, in which the anatomical conditions were the same, the *Bacillus prodigiosus* was not recovered in the intestine, although it was readily isolated from the gall bladder. The third test resulted positively. The menstruum used in the various experiments was 0.8 per cent. salt solution; in this last inoculation, however, rabbit serum was substituted.

From these experiments it was evident that, although the bacteria were excreted in the bile, they had gained access to the lumen of the intestine by some other route. Accordingly in the subsequent series of experiments not only was the bile duct ligated, but the pancreatic duct as well. This duct enters the small intestine about 12 inches below the pylorus, and, with this knowledge and a little experience, can readily be found and ligated just before it pierces the intestinal wall. Five experiments of this description were performed, all essentially alike, except for a difference in the quantity of culture material injected, and of the interval which elapsed between the time of injection and the killing of the animal. I shall not detail the manner in which the cultures were obtained from the intestinal contents, as this technic is fully explained in the

previous article on antiperistalsis.[5] It is, I believe, sufficient to repeat that the cultures were taken under the strictest precautions to avoid contamination by means of blood; to this end the intestine was seared through all its coats until it had the consistency of parchment, and was then incised by means of a heated scalpel. Of the five experiments in which both the ducts were ligated, all gave a positive result; that is, the test bacillus was recovered to some degree from the small intestine. Of these tests the most exacting were two (Experiments 24 and 25) in which only one loop of culture material was inoculated, and only one hour was allowed to elapse before the animal was killed. For the details the tabulated data may be consulted. It will be seen that the pancreatic duct does not seem to play an important rôle in the excretion of bacteria under the conditions imposed; it seemed a matter of indifference whether or not it was ligated.

The possibility had to be considered as to whether the bacteria, having reached the lungs by way of the blood, had ascended to the pharynx and in this way entered the gastro-intestinal tract. In order to decide this question two experiments were undertaken in which this path of entrance, as well as that by way of the bile duct or the pancreatic duct, was excluded. To this end the duodenum was divided between ligatures just below the entrance of the bile duct, and in addition the pancreatic duct was ligated as in the previous experiments. Thus the stomach and upper alimentary tract were severed from almost the entire intestine. The bile still flowed into the upper duodenum, as the duct had not been ligated. This arrangement possessed the advantage of not causing a damming back of the bile, which in all the previous experiments was a factor which existed, and which might be thought to have affected the results. This technic was carried out on three rabbits. The animals recovered one to two hours after the operation, and were then given an intravenous inoculation of *Bacillus prodigiosus*. The details are recorded in the experimental data (Experiments 26, 27 and 28). They may be summarized by stating that in one instance the result was absolutely negative; that is, the bacillus was not recovered, but that in the two other experiments the *Bacillus prodigiosus* was cultivated from the small intestine below the ligature. The bacilli evidently were not numerous, and they did not seem to be confined to the contents of any one section of the small intestine.

The only possible portal of entry into the intestine in this last series of experiments seemed to be the wall of the intestine itself, as all other paths had been barred. In order to exclude the complicating factor of shock, an experiment was undertaken in which the animal was allowed a longer period of recovery. To this end an intestinal fistula was made in the dog. The operation was kindly performed by Dr. Ernest Sachs, who sutured a loop of the duodenum into the abdominal wall, and in this way made a fistula with a double opening. The upper segment received the stomach contents, the bile and the pancreatic juice. For two days the dog was fed through the fistula and did well, although loss in weight was evident. After this interval the abdominal fistula was closed; this was accomplished by inserting a tube into the opening of the upper as well as into the opening of the lower loop of intestine, and then clamping them off. The physiological condition of the animal from our point of view now resembled that of the rabbits described above, in which the intestine was severed just below the bile duct. The only possible remaining path of communication between the blood current and the lumen of the intestine was the intestinal wall. An inoculation of four "loops" of *Bacillus prodigiosus*, suspended in 4 c. c. of salt solution, was made into the jugular vein. The dog was chloroformed to death two hours later. As the result of this experiment is given in full detail in a table (Experiment 29), it is unnecessary to do more than to summarize here. The table shows that a large number of cultures were taken from various parts

[5] Hess, A. F.: Antiperistalsis in its Relation to Tubercle Bacilli and other Bacteria in the Intestinal Tract, Jour. Med. Research, 1910, xxii, 129.

of the intestinal tract, and that from these tubes almost 100 agar plates were made. The *Bacillus prodigiosus* was demonstrated in the duodenum and in the upper and the lower ileum, but not in the cæcum or in the large intestine. From the number of negative tests it is evident that the test bacterium was not present in large quantities. This bacillus was isolated also from the urine, and was found in every instance in the gall bladder. It may be mentioned in this connection that almost all my experiments concurred in showing that the bile is the chief factor of excretion for bacteria given intravenously, and that the urine functionates in this capacity to a less extent.

It seems evident from the various experiments which have been summarized above that in the rabbit and in the dog bacteria are excreted from the blood directly through the intestinal wall. The manner by which this is accomplished, however, appeared by no means clear. Did the bacteria make their way unaided from the vessels through the intestinal mucosa, or were they carried into the lumen by means of phagocytes? As it seemed possible that they were carried through the wall by means of the leucocytes, an attempt was made to demonstrate this phenomenon. To this end, phenolphthalein suspended in water was injected intravenously into rabbits after the bile ducts had been ligated. An attempt was then made to recover this indicator from the intestinal contents. A large number of smear preparations were made from the mucosa of different parts of the intestine, after the contents had been rendered alkaline by means of sodium hydrate. In two such experiments, however, as well as in one in which carmin was used instead of phenolphthalein, no pink or red granules could be seen under the microscope. This fact is perhaps not surprising, considering the moderate degree of permeability which the intestinal wall was found to possess in regard to bacteria. I do not consider this question determined by these few experiments, and leave the question open as to whether bacteria traverse the intestinal wall by a simple passage through the vessels or whether they are carried by means of leucocytes.

CONCLUSION

As a result of my experiments it can be stated that bacteria, using the *Bacillus prodigiosus* for the test, are excreted from the blood, not only by way of the liver through the bile, and the kidneys through the urine, but also, to a less extent, directly through the intestinal wall. This was found to take place in one hour when one platinum loop of culture medium was inoculated. In these experiments all other paths of access from the blood to the lumen of the intestine were absolutely closed off, including the path from above by way of the pylorus, and the entry by way of the pancreatic and the bile ducts. It may be argued that the severity of the operative procedure contributed to the result by rendering the intestinal wall less resistant, and by increasing its permeability. It may be answered that this argument may be brought forward in the case of all experimental data. In the experiments performed the operations were carried out quickly, and with as little handling of the intestines as possible. Moreover, the question of complicating peritonitis or inflammatory reaction did not enter, as, apart from the careful asepsis observed, in almost all cases the animal was killed shortly after.

Although it is a well-recognized fact that bacteria can pass with the lymph stream through the intact intestinal mucosa, and then migrate from the lumen of the intestine to the blood, the reverse phenomenon, namely, that they pass from the blood through the mucosa into the intestine, has, as far as I am aware, not previously been demonstrated. I do not wish to correlate too strongly this phenomenon, demonstrated in the rabbit and in the dog, with conditions as they exist in man. It is interesting, however, to consider whether the analogy is applicable, whether the wall of the intestine functionates as an excretory organ not only in toxic conditions such as uremia, but also in bacteriemias such as typhoid fever or sepsis, and whether some of the intestinal symptoms and lesions,

manifesting themselves in these states, are brought about by what may be termed a mural excretion. This question opens up an interesting field of speculation for the clinician, and a point of view little considered by the pathologist and the physiologist.

NOTE.—Since the above article was written a short paper has appeared in the *Compt. rend. de la Soc. de biol.* (July 29, 1910, p. 181), entitled "Recherches sur l'Elimination du bacille d'Eberth et des Paratyphiques par l'Intestin," by L. Ribadeau-Dumas et P. Harvier, in which similar experiments are reported. The authors injected typhoid and paratyphoid bacilli intravenously into rabbits, having previously ligated and resected the bile ducts, and were able to recover the bacilli in the wall of the intestine and in the intestinal canal. The bacilli were cultivated from the appendix, duodenum and from the ileum; more readily in the wall than from the contents of the intestine. The data of the experiments are not given in detail, but the tests seem to have been numerous. The results confirm the conclusions which I have drawn from my experiments.

EXPERIMENTAL DATA

BACILLUS TUBERCULOSIS INOCULATED INTO RABBIT INTRAVENOUSLY. NO OPERATION

Experiment 13.—Weight of animal, 1,250 gm.; 130 mg. of a bovine culture suspended in normal salt solution injected into ear vein. Animal pithed after three hours. Positive inoculations: Blood 3, stomach 2, small intestine 2. Negative inoculations: Small intestine, 1. Remarks: The blood was caught in a solution of sodium citrate and inoculated intraperitoneally into guinea pigs. The contents of the intestine were filtered through gauze and allowed to settle before injecting it.

BACILLUS PRODIGIOSUS INOCULATED INTO RABBIT INTRAVENOUSLY. NO OPERATION

Experiment 17.—Weight of animal, 1,800 gm. Three loops of culture material injected. Animal pithed after two hours. Positive cultures: Duodenum 4, middle ileum 4, lower ileum 1. Negative cultures: Stomach 4, lower ileum 3.

BACILLUS PRODIGIOSUS INOCULATED INTO RABBIT INTRAVENOUSLY. PYLORUS LIGATED

Experiment 14.—Weight of animal, 2,140 gm. Three loops of culture material in 2 c. c. of salt solution injected; two-hour interval between operation and inoculation; one hour and three quarters between inoculation and death by chloroform. Positive cultures: Duodenum, 4. Negative cultures: Duodenum 6, middle ileum 7, lower ileum 5. Remarks: Of the 22 tubes containing 30 c. c. each of bouillon, only four showed *prodigiosus;* 0.5 c. c. of intestinal contents were transferred. The contents of the duodenum were found to be almost sterile.

Experiment 15.—Weight of rabbit, 1,520 gm. Three loops of culture in 1 c. c. of salt solution inoculated; animal chloroformed two and a half hours after inoculation. Positive cultures: Duodenum 2, lower ileum 2. Negative cultures: Duodenum 4, middle ileum 4.

BACILLUS PRODIGIOSUS INOCULATED INTRAVENOUSLY INTO RABBIT. COMMON BILE DUCT DOUBLY LIGATED AND SEVERED BETWEEN LIGATURES

Experiment 18.—Weight of rabbit, 1,850 gm. Three loops of culture inoculated one hour after operation; animal chloroformed to death two hours later. Positive cultures: Duodenum 3, upper ileum 1. Negative cultures: Stomach 5, duodenum 7, upper ileum 4, lower ileum 5.

Experiment 19.—Weight of rabbit, 2,050 gm. Same amount of material inoculated and same intervals observed as in Experiment 18. Positive cultures: Bile from gall bladder. Negative cultures: Duodenum 6, upper ileum 6, lower ileum 6, stomach 6. Remarks: In this experiment the bile duct was ligated, but not incised.

Experiment 20.—Weight of rabbit, 1,900 gm. Two loops of culture suspended in rabbit serum inoculated three hours after operation; same interval as previously between inoculation and killing of animal. Positive cultures: Upper ileum 3, gall bladder. Negative cultures: Stomach 5, duodenum 5, upper ileum 2, lower ileum 5.

BACILLUS PRODIGIOSUS INOCULATED INTRAVENOUSLY INTO RABBIT: DOUBLE LIGATION OF COMMON BILE DUCT AND PANCREATIC DUCT.

Experiment 21.—Three loops of culture inoculated into rabbit which was killed three hours later; double ligation of common bile duct and pancreatic ducts. Positive cultures: Duodenum, upper ileum. Negative cultures: Lower ileum. Remarks: Details are missing as regards the number of cultures taken.

Experiment 22.—Repetition of previous experiment except that rabbit was killed one and one-half hours after inoculation. Positive cultures: Duodenum, 2. Negative cultures: Duodenum 4, ileum 6.

Experiment 23.—Two loops of culture inoculated. Rabbit killed after two-hour interval. Positive cultures: Common duct 3, duodenum 2, ileum 3. Negative cultures: Colon 5, duodenum 3, ileum 2. Remarks: Six loopfuls of the contents of the intestine were used for the transfers to the bouillon tubes.

Experiment 24.—One loopful of culture inoculated. Animal killed after interval of one hour. Positive cultures: Lower duodenum 2, ileum 1. Negative cultures: Upper duodenum 4, lower duodenum 3, ileum 5.

Experiment 25.—This experiment was the counterpart of Experiment 24, except that both ducts were incised as well as ligated. Positive cultures: Upper duodenum, 4. Negative cultures: Lower duodenum 9, upper duodenum 5. Remarks: For these experiments 0.5 c. c. intestinal contents was transferred to each tube of broth. Agar plates made from these tubes 24 hours later showed numerous colonies of *Bacillus prodigiosus.*

CULTURES FROM DUODENUM ABOVE FISTULA			CULTURES FROM DUODENUM BELOW FISTULA		
Tube No.	Amount of Intestinal Contents Used	Result* Colonies	Tube No.	Amount of Intestinal Contents Used	Result* Colonies
1	1 loop	N	1	3 loops	N
2	3 loops	P Many	2	5 loops	N
3	5 loops	P Few	3	7 loops	P 1
4	7 loops	P Few	4	9 loops	N
5	9 loops	P Many	5	12 loops	N
6	0.1 c.c.	P Many	6	0.25 c.	P 4
7	0.2 c.c.	P Many			2
8	0.3 c.c.	P Many	7	0.5 c.c.	N
9	0.4 c.c.	P Many	8	0.75 c.c.	N
10	0.5 c.c.	P Many	9	1 c.c.	P 4
					5
			10	2 c.c.	P 3
					6

*P=Bacillus prodigiosus. N=Negative.

CULTURES FROM UPPER ILEUM			CULTURES FROM LOWER ILEUM		
Tube No.	Amount of Intestinal Contents Used	Result* Colonies	Tube No.	Amount of Intestinal Contents Used	Result* Colonies
1	3 loops...	N	1	3 loops...	N
2	5 loops...	N	2	5 loops...	N
3	7 loops...	N	3	7 loops...	N
4	9 loops...	N	4	9 loops...	N
5	12 loops...	N	5	12 loops...	P 2
6	0.25 c.c...	N	6	0.25 c.c...	N
7	0.5 c.c....	P 2 5	7	0.5 c.c....	P 4 4
8	0.75 c.c...	N	8	0.75 c.c...	N Overgrown
9	1 c.c.....	P 11 3	9	1 c.c.....	N Overgrown
10	2 c.c.....	N Overgrown	10	2 c.c.....	P 3 6 Overgrown

CULTURES FROM CECUM			CULTURES FROM LARGE INTESTINE		
1	1 loop.....	N	1	1 loop.....	N
2	2 loops.....	N	2	2 loops.....	N
3	3 loops.....	N	3	3 loops.....	N
4	4 loops.....	N	4	4 loops.....	N
5	5 loops.....	N	5	5 loops.....	N

CULTURES FROM GALL-BLADDER			CULTURES FROM URINE IN BLADDER		
Tube No.	Amount of Bile Used	Result* Colonies	Tube No.	Amount of Urine Used	Result* Colonies
		2	1	3 loops.....	N
1	3 loops.....	P 4	2	6 loops.....	N
2	6 loops.....	P 5 3	3	0.5 c.c.....	P 3 1
3	0.3 c.c.....	P 19 43			

For this entire experiment 56 bouillon tubes were inoculated and from these 112 agar plates were made 24 hours later. Two plates were made from each tube.

*P = Bacillus prodigiosus. N = Negative.

BACILLUS PRODIGIOSUS INOCULATED INTRAVENOUSLY INTO RABBIT AFTER DOUBLE LIGATION AND DIVISION OF DUODENUM AND LIGATION OF PANCREATIC DUCT

Experiment 26.—Previous to inoculation the duodenum was doubly ligated and divided just below the entrance of the bile duct. The pancreatic duct was also ligated. One loop of culture inoculated as soon as animal recovered from operation; one hour later animal chloroformed to death. Positive cultures: None. Negative cultures: Duodenum 10, ileum 5. Remarks: One, three and six loopfuls of intestinal contents were transferred to broth in this experiment and the two following.

Experiment 27.—One loop of culture material injected; rabbit chloroformed one hour after inoculation. Positive cultures: Duodenum above ligature 2, lower duodenum 2, ileum 1. Negative cultures: Duodenum above ligature 1, lower duodenum 3, ileum 4.

Experiment 28.—Repetition of Experiment 27. Positive cultures: Duodenum above ligature 3, lower duodenum 3. Negative cultures: Duodenum above ligature 2, lower duodenum 9, ileum 5.

BACILLUS PRODIGIOSUS INJECTED INTO JUGULAR VEIN OF DOG

Experiment 29.—Intestinal fistula made below entrance of bile duct and pancreatic ducts. Animal fed through fistula for 48 hours, and starved 12 hours preceding inoculation. Ends of fistula closed off. Four loops of culture in 4 c. c. of salt solution injected. Dog chloroformed two hours later.

PURE CULTURES OF AMŒBÆ FOUND IN INTESTINES OF MAMMALS

Anna W. Williams, M. D.

Introduction.—In February of this year the writer published the results of growing on brain-streaked agar a pure strain of an amœba isolated in pure mixed culture from a case of human amœbic dysentery.[1] The present paper is a report of the continuation of the study of pure cultures of amœbæ. It presents the results of growing in pure cultures several strains of amœbæ obtained from the intestines of different mammals. The culture media used were crushed brain, liver, kidney, and other tissues of rabbit or guinea pig, streaked on nutrient agar.

It seems desirable, since there is some ambiguity in the statements of several of the writers on the subject of growing amœbæ in pure cultures, to give first a short critical review of the previous reports.

Critical Review.—We find in the literature three meanings to the term "pure culture" as applied to amœbæ. First, a culture of amœbæ is called pure when it is grown free from other protozoa, though any other living micro-organisms, such as bacteria and yeasts, may be present. This meaning is given more frequently in the older reports (Cunningham,[2] 1881; Celli and Fiocca,[3] 1895, and others); still, even at present, some writers continue to use the term in this, to say the least, non-bacteriologic sense (Saul,[4] 1904; Thomas,[5] 1906, etc.). Second, "pure mixed" cultures, that is, cultures of a single strain of amœba with a single species of bacterium, are frequently and wrongly called pure cultures. Third, a single species of amœba growing in successive culture generations without the presence of other living organisms is rightly called a pure culture; and, as it is in this sense that the term pure culture is used in this report, the work of only those investigators who have given this meaning to the term will be reviewed.

Amœbæ have been cultivated in mixed cultures more or less successfully since 1856 (Auerbach[6]). Much later (1898) successful "pure mixed" cultures were made by Frösch[7] and by many following him. Full bibliographies have been given by Musgrave and Clegg,[8] Walker[9] and others on parasitic amœbæ and their saprophytic relatives, and very large bibliographies they make; but in the whole list are found only four writers who claim to have obtained amœbæ growing in strictly pure cultures.

1. Kartulis[10] in 1893 said that he obtained by growing in sterile hay infusion a pure culture of amœba from a bacteria-free liver abscess; but he did not mention culture generations, and in 1900 in an article on tropical dysentery[11] he ignores his earlier statement and says, "Parasitic amœbæ are seldom cultivated and then in impure cultures." He is still quoted, however, as obtaining pure cultures of amœbæ.

2. Casagrandi and Barbagallo[12] in 1897 said that they obtained once a strictly pure culture of amœba (Amœba spinoza) a saprophyte, on alkaline, 5 per cent. fuccus crispus medium; but these authors also say nothing about culture generations, and nothing further about technic.

3. Tsugitani[13] in 1898 tried to grow three strains of saprophytic amœbæ with several species of killed cultures of bacteria and he found that one strain grew with one species of dead bacteria. Nobody has been able to corroborate his work, though many others have tried to grow amœbæ with killed cultures of bacteria. He also says nothing about culture generations. It seems, however, in the light of some work Miss J. T. Chase and the writer have been doing with killed cultures as food that Tsugitani probably obtained in this way a continued growth of his amœba. We have grown one strain of amœba for ten culture generations on agar cultures of dysentery bacilli killed by heating in normal salt solution for two hours at 60° C. Though we obtained undoubted growth, it had to be helped along, and was, at best, a very slow and scanty one.

We see from the above review that no one has reported a continued growth in successive culture generations of pure cultures of amœbæ. While, on the other hand, Musgrave and Clegg,[14] 1906; Walker,[9] 1908; Craig,[15] 1908, and the majority of investigators who have tried to grow pure cultures of amœbæ all make emphatic statements as to the impossibility of obtaining such cultures by any methods tried.

The conclusions of Musgrave and Clegg[14] are interesting as showing how near these authors came to the using of tissue cells as a medium. They say: "Pure cultures of amœbæ which will continue to propagate in media free from other living micro-organisms have not been obtained. Amœbæ from liver abscesses and the intestines would not thrive in various filtered extracts from human and animal livers, although these proved excellent culture media for bacteria. Liver and other animal tissue extracts obtained in a sterile manner furnished no better results than the filtered products. The work of all recent authors as well as our own seems to point to the impossibility of such a procedure."

Walker[9] says: "These cultures of bacteria-free amœbæ . . . grew when transplanted on fresh media only when supplied with a living culture of bacteria." Again: "Amœbæ will not grow apart from bacteria or other micro-organisms upon which they probably feed instead of obtaining their nourishment directly from the culture medium. Therefore, pure cultures in the bacteriological sense are not possible."

In looking carefully over the literature the writer has found that no one apparently has tried crushed tissue cells as a medium; though, considering the number of bacteria-free liver, brain, and lung abscesses that have been reported it seems rather strange that the use of such a medium has not occurred before to some worker.

It is true that a number of writers have recognized the probability of amœbæ using tissue cells in some way as food. Thus, Councilman and Lafleur[16] (1891) say: "No micro-organisms other than amœbæ were found in the smallest and most recent abscesses . . . and even in the large the bacteria which were found did not seem to have any causal connection with the abscess. None of the organisms which we are accustomed to consider pus organisms were found. The lesions were of a different character from those produced by bacteria."

Kruse and Pasquale[17] (1894) say: "We are not of the opinion of Kartulis that the amœbæ are simply carriers of the pus-forming bacteria into the liver, but we believe that, as in the ulcerated intestinal wall, the amœbæ have a direct action in the degenerative process. On the other hand, we do not believe with Councilman and Lafleur that the existence of bacteria in liver abscesses and intestinal ulcers is a non-specific complication or an entirely unrelated phenomenon; because the presence of bacteria is far too constant. . . . Even in 'sterile abscesses,' there are probably a few bacteria."

Musgrave and Clegg[14] (1906) say: "In all intestinal lesions and in many liver and lung abscesses there is present, in addition to amœbæ, one or more varieties of bacteria, and cultural experiments demonstrate that under these circumstances a definite bacterial symbiosis often exists between the amœbæ and some of the organisms which are present, whereas at the same time the amœba exists in partial tissue symbiosis. In other instances, even in mixed amœbic and bacterial lesions, the association of the bacteria seems no longer to be necessary for the life and action of the amœbæ, for no symbiosis with any of the bacteria which are present can be found by cultural methods. It is probable that, in these cases, the amœbæ have taken on a symbiosis with the animal tissues; in other words, have become true parasites and that the presence of the bacteria is without significance."

Lesage[18] also came close to the point of using tissue cells as a culture medium. He recommends his medium especially for the isolation and growth of "Entamœba histolytica" from abscesses of liver or intestines in amebic dysentery. He obtained a leucocytic exudate (that from the guinea-pig best), placed it in the ice-box for one day and then centri-

fugalized. The supernatant liquid was the culture medium. In this medium the whole contents of a liver abscess were placed. If intestinal contents were used they were first put into the peritoneal cavity of the guinea-pig to lessen the number of bacteria. The amœbæ developing in this culture medium, the author says, have all the characteristics of Entamœba histolytica as described by Schaudinn. The cysts in pus from old liver abscesses opened in this medium after two to three days at room temperature. The liberated amœbæ developed into adult amœbæ which, after a short period, produced new cysts. Lesage says nothing about culture generations, neither does he describe his amœbæ minutely, so we can only infer that he obtained the usual one cycle of development which is often obtained with encysted amœbæ on many kinds of culture media. His medium was not bacteria-free.

Original Work.—In a problem of this kind where the claim put forward has been disallowed so strongly by so many good workers, and where error undetected may so readily creep in, it seems necessary to give a more minute description than usual of the whole work.

First in regard to the strains employed 1. "Amœba coli." This culture was obtained through Dr. G. N. Calkins in 1906, who received it the year before directly from Musgrave's Laboratory. It is the one Musgrave and Clegg [8] labeled 11,524 and with which they performed many of their experiments. At the time it came into the writer's possession it had been in a sealed agar plate for a year after growth with cholera bacillus. After moistening this dried culture with sterile normal sodium chloride solution, transplants on protozoan agar were made from it on which, after four days, a certain number of cysts opened and some motile amœbæ appeared. The culture used in this work is the one hundredth culture generation from a single amœba isolated from one of the early transplants. The original culture was obtained by Musgrave and Clegg from the feces of a case of amœbic dysentery in man. Whether it is Entamœba histolytica or whether it was pathogenic is not definitely proved, though Musgrave and Clegg's experiments are certainly strong evidence in favor of its pathogenicity.

2. "Amœba limax." This amœba was isolated from potato parings and tap water, in 1906, by M. A. Wilson of this laboratory. It was identified as one of the limax group by Dr. Calkins, who studied some phases of its life cycle with the writer.[19] It had been carried on with a yellow air bacillus and with B. dysenteriæ (Shiga type) since its isolation, and was in its one hundred and tenth culture generation at the time of these experiments.

3. Two strains of tiny amœbæ, size one to twelve microns, cysts two microns, isolated several times in succession in 1907 from feces of two cats respectively. They grow very rapidly, use up the bacteria (coli-typhoid group), and encyst quickly. They have to be transplanted every two days at room temperature if motile amœbæ are required. These organisms are interesting because their cysts are formed exogenously, as Entamœba histolytica is said to form its cysts.

4. "Amœba intestinalis" sent the writer by Walker in 1906; described by him as a new species [9]; carried on by writer with B. dysenteriæ; in its ninety-sixth cultural generation at the time of these experiments.

5. "Amœba cobayæ" sent by Walker in 1906; described by him as a new species[9]; in ninetieth cultural generation at beginning of this work.

6. One strain of amœba from dog similar to Amœba intestinalis.

All of these were grown, as routine, with the dysentery bacillus (Shiga) on nutrient agar one per cent. acid to phenolphthalein, and were transplanted every seven to thirty days. All of the above strains with the exception of Amœba limax were presumably parasitic, in a certain sense at least, in the animals from which they were isolated. Whether any of them were capable of becoming tissue parasites is a question which the present work may help to clear up. This will be discussed later.

Obtaining amœbæ free from other living organisms.—Two methods have been employed, differing only in detail from those successfully used

by other investigators. The first method, that of transferring motile amœbæ from the zone beyond the growth of the bacteria, was reported in the first paper. The second method was used with those strains of amœba that digested the bacteria quickly, that is, with strains numbered 3, 4, 5, and 6. These strains under certain condition in from one to two weeks grown on nutrient agar, apparently digest all of the accompanying bacteria (B. dysenteriæ) and remain finally a mass of cysts. These cysts, transplanted on nutrient agar without bacteria, remain in large part encysted. A few cysts may open, but the freed amœba soon encysts again unless suitable food is within reach. In order to be sure that no bacteria have been carried over with the amœbæ the new transplants are allowed to remain, some at 36° C. and others at room temperature, for twenty-four to forty-eight hours. If no bacteria show themselves in this time it is reasonably certain that none have been carried over. The control plates are allowed to remain longer. The nutrient agar (one per cent. acid to phenolphthalein) used in most of the work allows contaminations readily to show themselves. The first method, that of transferring motile amœbæ, is to be recommended in most instances.

Obtaining sterile tissues for food.—Several different organs of guinea-pigs and rabbits and the brains of dogs have been used. The animals are killed by chloroform. Their bodies are soaked for ten minutes to half an hour in five per cent. carbolic acid. The organs are removed as quickly as possible in a room practically bacteria-free. Different sterile instruments, well sharpened, are used for each slip. The abdomen is opened, the periphery of the liver is cut off and placed on a freshly made agar plate; then the spleen, the kidneys, and any other tissue needed are rapidly cut out and put each on a separate plate, and, lastly, the cerebrum is removed in the same manner. The tissues are now cut into tiny pieces with sharp sterile scissors, and are taken up with sterile forceps and spread over several freshly made agar plates. These plates are then placed in the thermostat (36° C.) for twenty-four hours. With all these precautions for insuring sterility there is no need of washing the freshly removed tissues first in five per cent. carbolic acid, as was recommended in the first paper. Emulsions of liver and brain in sterile neutral glycerine have given a moderately good result any time up to three weeks. They have not been tested later, neither have they been tried for more than three successive culture generations. These glycerinated emulsions of tissue were always allowed to remain for twenty-four hours in the thermostat spread on freshly made agar plates, before being planted with the amœbæ. This allowed the excess of glycerine to be absorbed into the agar.

Growth on sterile tissue media.—The broken up tissue, fresh or glycerinated, after being in the thermostat for twenty-four hours is ready to add to the bacteria-free amœbæ. A small quantity of the tissue is gently mixed up with the amœbæ on each agar plate, and the plates are placed some at 36°C. and others at 20-24° C. (room temperature). If the thermostat cultures show slight or no growth in twenty-four hours, a loop or two of sterile 0.8 per cent. sodium chloride solution may be added.

Usually in twenty-four hours at 36° C. or in three to five days at room temperature, with certain strains of amœbæ, there is an abundant growth on brain, liver, and kidney tissue prepared in this way.

In order to rule out the presence of contaminating organisms, especially of those difficult to demonstrate or of those growing very slowly, many control plates were made, at first with each transfer, later at less frequent intervals. The controls spoken of in the first paper—blood, broth, normal salt solution, plain agar—have been used, and others also, such as various crushed tissues which gave no growth with the amœbæ. Then, as an added control—and a very important one—three sets of spreads have been made at practically each transfer, one stained by Giemsa's method, one by Heiderheim's, and the other with fuchsin-methylene blue mixture.

"Amœba coli," Amœba intestinalis, Amœba cobayæ, and the strain from the dog all grow on normal brain tissue with the greatest ease. Amœba coli is now in its fifty-fourth culture generation on brain tissue, transplanted every two to four days at 36° C., since December 31, 1910, and in the twentieth culture generation at room temperature transplanted every one to four weeks. Amœba intestinalis, started later, is in its thirtieth culture generation at 36° C. Amœba cobayæ was carried on for fifteen culture generations, and the strain from dog's intestines for ten culture generations. Amœba coli is in its thirty-fifth culture generation on kidney and in its thirtieth culture generation on liver; Amœba intestinalis is in its sixteenth culture generation on liver and on kidney, both at 36° C. The attempts to grow these two strains with lymph nodes, suprarenals and spleen have so far given negative results, but the attempts have not been many. All of the many efforts to grow Amœba limax and the two tiny amœbæ on crushed brain tissue have given negative results. No attempt has been made to grow them on other tissue. Amœba coli has been grown successfully on dog's brains for five culture generations.

The points which have been considered in the study of pure cultures of amœbæ on tissue media are the following:

(1) Temperature, (2) rate of growth, (3) morphology and life cycle, (4) pathogenesis, (5) isolation from cases of human dysentery, (6) action of amœbæ on diseased tissues in which no specific micro-organisms have been positively recognized.

1. Temperature.—Most students of parasitic amœbæ state that these organisms grow best at room temperature. The truth of this statement depends upon the definition of the word best. Walker[9] says: "Cultures of parasitic and of free-living amœbæ will grow on artificial media at room temperature or at 37° C., but they maintain their growth and vitality longer at room temperature." He does not call attention to the fact, however, that they grow much more slowly at room temperature than at 37° C. At the latter temperature, according to the writer's experience, all amœbæ with proper food grow very rapidly and abundantly, therefore, if rapidity and abundance are the qualities which constitute goodness in a culture, growth for these strains is best at thermostat temperature.

The pure tissue cultures grow even more quickly and abundantly than the pure-mixed cultures at 36° C. Cultures at this temperature must, of course, be transferred more frequently than at room temperature, usually every two to four days, while the room temperature cultures need not be transferred under a month. The thermostat cultures, it is true, encyst sooner than the room cultures and the individual transplant loses its vitality sooner, but frequent new transplants at 36° C. show no loss of vigor, on the contrary, they seem to have increased ability to multiply rapidly. Lasting cysts are better produced at the lower temperatures (20-25° C.) on thinly poured, slightly nutritious culture media that quickly dries after cysts are formed.

Only the two temperatures, 36° C. and about 22° C., have been tried by the writer. It is possible that 30° C., or thereabouts, might prove better for maintaining most characteristics of amœbæ.

2. Rate of growth in cultures.—This varies with temperature, condition and kind of tissue media, age of transplant, stage of development, species, and adaptation. The effect of temperature has just been noted. The age of transplant and stage of development which usually go hand in hand have a good deal to do with rate of growth at the beginning. Thus, an encysted culture will develop in a period increasing with the age of the cysts, while transplants of motile amœbæ on fresh tissue media are checked but slightly in their growth, especially if many be transplanted, *i. e.*, a small platinum loopful. Occasionally, however, for unknown reasons, the new transplants encyst, if motile, or remain encysted unless they are stirred or after twenty-four hours a loop of normal sodium chloride is added to the culture.

Growth is somewhat more rapid and abundant on tissue freshly isolated, or kept in ice-box for a short time than when kept in the thermostat first for twenty-four hours. If perfectly fresh tissue is used it must be most carefully controlled for the presence of extraneous organisms. On the whole, these amœbæ grow more quickly and abundantly on brain media than on kidney or liver media, and they grow better on kidney than on liver media. Strains freshly isolated from pure-mixed cultures with bacteria do not grow as well as after several transplants.

3. Morphology and life cycle.—So far only a few interesting points concerning this topic have been noted; interesting, because they contradict several statements given by various authors in regard to the differential diagnosis of pathogenic and non-pathogenic amœbæ in human beings.

The observations on only two of the strains of amœbæ—Amœba coli from human amœbic dysentery and Amœba intestinalis from normal dog's intestines—will be reported at present.

Before beginning the report of the personal study, the question of the effect on morphology and life cycle of amœbæ when grown on artificial culture media should be considered. The general criticism is that "artificial cultures" grow atypically, but just the nature and importance of this "atypical" growth in any instance have not been made clear. Granted that in an occasional individual case fundamental changes may occur on artificial media, in general, changes under these conditions must be quite superficial or we should have to change our ideas in regard to the frequency of mutation. Such changes must be only transitory variations, and species characteristics should be looked for in those appearances which are similar under several conditions of environment. For instance, in amœbæ the pseudopods are known to be decidedly variable within limits, therefore until we know by sufficient controls what these variations are we should not use size and shape of pseudopods as species characteristics. The same is true for motility of organism as a whole, as well as for its size, shape, color, number of food vacuoles, position of nucleus, distinction between cytoplasm and endoplasm, contained bodies, such as red blood cells, and, lastly, for its staining reactions. Peculiarities in all of these characteristics have been emphasized as aids in differential diagnosis. Thus Craig[15] says: "If, in a freshly voided specimen of feces we observe large, motile amœbæ, showing a clear hyaline ectoplasm, the distinction between the ectoplasm and endoplasm being marked, an absence of a nucleus, or a nucleus situated near the periphery of the endoplasm, small in size, poor in chromatin, and having a dimly defined nuclear membrane with two or more vacuoles, and with or without red blood corpuscles in the endoplasm, we may be sure that the organism observed is Entamœba histolytica;" also, 'In Entamœba coli the ecto- and endo-plasm are both grayish in color and there is never observed the greenish color which is not uncommon in Entamœba histolytica. In Entamœba histolytica the endoplasm is very often observed to contain one or several red blood corpuscles, while in Entamœba coli red blood corpuscles are very, very rarely observed in the endoplasm." Further, "In well-stained preparations the two species of amœba can be distinguished by the difference in the staining reactions of the ecto- and endo-plasm. In Entamœba histolytica the ectoplasm stains more intensely with Wright's stain than does endoplasm, while in Entamœba coli the opposite is true.

Not one of these characteristics taken separately, nor all of them together, can be considered by the writer, in the light of the present study, as sound differential points, since amœbæ from two such widely separated habitats as Amœba coli and Amœba intestinalis, and showing certain constant morphological differences, vary so much on tissue media in regard to these characteristics.

Size.—On the whole the size of the amœbæ varies more than it does in cultures growing with bacteria, particularly at 36° C. Amœba intestinalis is on the average slightly larger than Amœba coli. The largest forms

measured are about fifty microns, the average of the majority about twenty-five microns, while of the many small forms (produced by budding) the smallest measure about two microns. We see then that these two strains, which are apparently different from each other, and one of which, at least, is probably not Entamœba histolytica, both may show in artificial culture media many forms similar in size to those described for Entamœba histolytica and therefore could and presumably would show such forms when grown in the intestines. Hence, this point, at present, seems not to be of worth in differential diagnosis.

Shape.—Both forms are round or slightly oval when at rest. Their pseudopods are quite variable in shape, depending upon the consistency of the medium and the age of the culture. Those of Amœba intestinalis are markedly lobose in any partially soft media which allow the amœbæ to penetrate. The pseudopods under these circumstances are often long and narrow or finger-shaped, corresponding to those described for Entamœba histolytica in feces. This is probably due to the fact that the conditions of growth as to consistency of media are somewhat similar. On solid media where the organisms remain on the surface or in more fluid media, under both of which conditions the organisms meet with less resistance in their progress, the pseudopods are apt to have spinous processes. In Amœba coli the spines are more pronounced than in Amœba intestinalis. Several spines usually pass out from a broad basal pseudopod. The pseudopods of Amœba intestinalis are more refractive than those of Amœba coli. Both of these species vary in motility according to age, culture media, and temperature. Young cultures (twenty-four hours old) in fluid culture media, at higher temperature (36° C.) are most motile. On the whole, Amœba coli is less motile than Amœba intestinalis. In cultures which have been constantly kept at 36° C. motility ceases in a short time when put at room temperature, to be resumed after a varying period according to the age of the organisms. This is true for all cultures kept at 36° C. Some species resume movements more quickly than others, for instance Amœba limax and the tiny amœbæ which are all very motile in young cultures on practically any medium.

Cystoplasm.—The cystoplasm in both Amœba coli and Amœba intestinalis shows distinct separation into ectoplasm and endoplasm in the large forms when the organisms are in motion in a more or less fluid medium or on moist surfaces, but in media which they can penetrate this distinction is not so apparent. Thus Amœba coli in brain media often sends out spinous pseudopods into the granular brain food, in which the spines seem to become lost, so even in well fixed and stained specimens the border line between amœbæ and food medium is not well defined. The reticular cytoplasm of such an organism seems simply to merge into the granules of the brain medium. The reticulum and cell contents show distinctly about the nucleus.

With both Giemsa and fuchsin-methylene blue stains the cytoplasmic reticulum is clearly demonstrated in these pure growths, since there are no bacteria to help false interpretations. With the former stain the cytoplasm of Amœba intestinalis takes a dark blue or purple, the ectoplasm staining more intensely than endoplasm, which, Craig claims, is true for Entamœba histolytica; while with this stain the ectoplasm of Amœba coli takes the stain more faintly than the endo plasm, that is, as Craig says, Entamœba coli stains.

The more refractive pseudopods of Amœba intestinalis appear finely granular under the high power (x 1,500), while the pseudopods of Amœba coli are more hyaline.

At 36° C. both of these species generally show one to very many vacuoles, some large, others very small, sometimes one or two very large ones with none or several small ones. The largest amœbæ contain the most vacuoles. Amœba limax at 36 C. may also show many vacuoles, therefore the presence of many vacuoles and large size seem not to be of value as a diagnostic point.

In regard to the importance in differential diagnosis of the presence of red blood cells within the amœbæ, the present study proves conclusively that it also is not great. For all of the strains studied in this connection (Amœba limax, Amœba coli, and Amœba intestinalis) are markedly phagocytic for erythrocytes when adult motile amœbæ are planted in fluid or moist fresh culture media containing fresh blood cells and examined in about twenty-four hours at room temperature. At this time a variable number of amœbæ are found containing one to many red blood cells. Of the three species studied Amœba intestinalis is the most phagocytic. Some individuals may be so packed that the number of contained erythrocytes cannot be determined. Amœba limax is the least phagocytic of these three; usually one to four blood cells are seen in occasional amœbæ but a few times as many as eight have been counted.

After twenty-four hours a rapid digestion of the contained erythrocytes is evident, hence the cultures must be examined in about that time, or somewhat earlier if kept at 36° C. After forty-eight hours there is a marked diminution of the number of free red blood cells, and none, or only remnants are found in the amœbæ, a number of which have encysted; and both motile amœbæ and cysts may have a decidedly greenish color. This process occurs both in pure and in pure-mixed cultures. Though the amœbæ apparently digest the red blood cells they do not seem to find in the latter sufficient if any food for continued life, since amœbæ planted in blood alone or in blood added in varying quantities to broth or agar do not propagate after the first or second culture generation. There seems to be some increase in the phagocytic power of amœbæ for erythrocytes after growing for several successive culture generations in suitable media containing fresh red blood cells.

Reproduction.—The nuclear changes and the methods of reproduction observed do not in general differ from those seen by the writer in pure-mixed cultures. Budding is a little more frequent. The nucleus is more varied in chromatin content and in position, but divisions occur by definite mitoses, and cysts develop essentially as they do in the latter cultures. Budding is comparatively more frequent than mitoses at thermostat temperature, while the opposite is true at room temperature.

The process of the separation of small portions or "buds" from the parent amœbæ has been repeatedly observed under the microscope and corroborated by spreads stained with Giemsa. The separated buds show a delicate blue reticulum dotted with small chromatin-staining granules which show distinctly only in well fixed, well stained spreads. Many different sized buds may be seen in some young cultures, but the small ones predominate. Under favorable conditions some of the larger of the separate buds encyst, which process somewhat resembles that described for encystment of Entamœba histolytica.

No definite evidence of endogenous budding or spore formation has been obtained. The large rounded chromatin staining masses so often seen within the cytoplasm of the amœbæ in bacteria-mixed cultures do not appear in these pure cultures. In their places are morphologically similar masses taking a light blue stain with Giemsa, or a reddish stain with fuchsin-methylene blue. The probable interpretation is that these masses are more or less digested food particles—bacteria in the pure-mixed cultures, and broken up tissue in the pure cultures.

Mitotic division is most frequently observed in young, vigorously growing cultures. Though in living individuals of both species the nucleus is often indistinct, in well fixed and stained specimens (spreads and impression slips fixed while moist) the mitotic changes may be clearly seen. Probably no amitotic division occurs unless the larger "budding" may be so considered.

The precystic, cystic, and postcystic stages require such prolonged, minute, and control study in order correctly to interpret the changes seen, that their description is left for a future report. We may state, however,

that from our morphological study we think the assumption fair that amœbæ isolated from mammals show essentially the same morphological changes in tissue culture media as they do in the intestines.

It is certainly true that the worth in differential diagnosis of the various characteristics described above, singly or taken together, is much less than others would have us believe. Indeed the recent demonstration of another species of amœba, presumably pathogenic for man, Amœba tetragena, and having many of the characteristics of Entamœba coli, has already thrown grave doubt upon the worth of these points in diagnosis.

It seems much more probable that Musgrave and Clegg and those agreeing are right in thinking that several different species of amœbæ produce or help produce human dysentery. And this brings us to the next point to be discussed.

4. Pathogenesis.—The fact that certain amœbæ may be nourished by tissue cells alone, as well as by bacteria alone, is strong evidence in favor of the view that amœbæ are of an importance equal to, if not greater than, bacteria in the production of amœbic dysentery and amœbic abscesses. It may be that in the living host the tissue needs first to be injured in some special way. The fact that pure cultures of "Amœba coli" have been inoculated into the liver and brain of two guinea-pigs and two rabbits with a negative result favors this idea. But the injury need not necessarily be directly from living bacteria.

These inoculations were made only after the fourth culture generation on brain tissue, so the amœbæ may not have become sufficiently accustomed to the brain media to produce positive results, or there may have been other controllable causes. That certain varieties of amœbæ, however, become strict tissue parasites seems from this study to be true. The question as to the nature of saprophytism and parasitism in the group of amœbæ needs much further study.

Pure cultures of the two strains, Amœba coli and Amœba intestinalis, were every day fed to two kittens, respectively, for four weeks. The growths on agar plates planted at varying times, thus allowing the mixture to contain cysts of different ages as well as motile amœbæ, were mixed in milk, and the dose was increased from one to several plates (4-8). The amœbæ were found in the stools after the sixth day. The kittens were first found to be amœba-free. The animal receiving Amœba intestinalis had several short attacks of diarrhœa without blood during the feeding. The one receiving Amœba coli had one short attack of bloody diarrhœa three weeks after the feedings were stopped. It apparently recovered in two days and both kittens are now well. The amœbæ in these stools seem to be quite similar to those in the pure cultures inoculated.

5. Isolation of cultures of amœbæ from cases of human amœbiasis.—Unfortunately, since the beginning of the present study, the writer has had no opportunity to work directly with human amœbic dysentery and therefore can only suggest that crushed-tissue media promise to be an aid in obtaining cultures of amœbæ from this disease, especially from the bacteria-free liver and other tissue abscesses which are so frequently a complication and in which the amœbæ have become accustomed to the tissue food. From these abscesses workers have so far had difficulty in obtaining growth of amœbæ. Even Musgrave and Clegg, who have reported more positive results than any one else, only obtained occasional growths in one or two plates out of many. In attempting growths from any one of these sources, especially from the intestinal contents, we must remember that it is not necessary at first to obtain bacteria-free cultures; but, since the bacteria present may easily overgrow the amœba, an attempt should be made to keep the former down. Probably the best results would be obtained with alkaline water-agar plates spread with small quantities of brain, liver or kidney tissue to which the amœbæ mixture should be added in small amounts. Many plates should be made, some kept at room temperature and others at 36° C. If no growth is seen in forty-

eight hours a loop or two of sterile normal sodium chloride solution should be added and the plates should be observed for some days further.

6. Action of amœbæ on diseased tissue in which no specific micro-organisms have been positively demonstrated.—The possibility of using amœbæ in pure cultures to aid in detecting micro-organisms that have so far eluded us led to the present work. It was called forth by Clegg's work on the lepra bacillus. The pure cultures have been tried so far only with rabies brains, though plans have been made to use them with material from measles, scarlet fever, and acute poliomyelitis. With rabies brains these amœbæ have grown well from the start; there seems to be no drawback to their growth either at room temperature or at blood heat, which is not always the case with normal brains used as food. Very little study has been made yet of these rabies brain cultures. They are virulent after a week at room temperature. Morphologically nothing definite has so far been demonstrated.

SUMMARY AND CONCLUSIONS

1. Successive pure cultures of certain strains of intestinal amœbæ may be easily obtained by using, as food, sterile brain, liver or kidney tissue freshly removed from the normal guinea-pig, rabbit or dog.

2. Morphologically the individual amœbæ in these cultures do not differ essentially from those of the same species growing in the intestines of mammals.

3. Certain characteristics—namely, large size, marked motility, clearly differentiated and highly refractive ectoplasm, nucleus poor in chromatin and situated near periphery of endoplasm, endoplasm showing two or more vacuoles and containing two to many red blood cells—which as a whole have been pronounced by others to be of use in differentiating pathogenic from non-pathogenic forms in the human intestines, have been shown by this study to be possessed by two species of amœbæ in pure culture, one at least, in all probability, non-pathogenic for man. Hence the diagnostic worth of these points is rendered doubtful.

4. Freshly removed tissue as food for amœbæ promises to be of aid in obtaining pure cultures of these organisms from cases of amebiasis, especially from bacteria-free amebic abscesses.

5. Pure cultures of amœbæ may be of use in helping to detect specific micro-organisms in infectious diseases of unknown origin.

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PREFACE TO VOLUME VII

The reports appearing in the present volume include studies carried on during the years 1912 and 1913, in the Divisions (Bacteriological, Preventive Medicine, Chemical) of the Bureau of Laboratories.

Since the original "Research Laboratory" has become merged into the larger "Bureau," it is thought best to replace the words "Research Laboratory" in the title of our volumes of Collected Studies, to "Bureau of Laboratories."

Most of the reports in this volume have already appeared in print, others are in press, and the few remaining are published only in this collection.

The statistical matter of the Bureau of Laboratories will be found, as in previous years, in the Annual Report of the Department of Health.

The recipients of this volume will confer a favor on their colleagues of the Bureau of Laboratories by sending their publications in exchange. These should be addressed to the Librarian, Bureau of Laboratories, foot of East Sixteenth Street, New York City.

W. H. P.

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In Memoriam

During the past year, the Research Laboratory has suffered a loss in the death, after a brief illness, of Dr. Ira Thompson Van Gieson.

Dr. Van Gieson was born at Tenant, N. J., forty-seven years ago, and lived for many years at Greenpoint, L. I., where his father is a well-known practicing physician. He was educated at Columbia University, and received his degree from the College of Physicians and Surgeons in 1885, after which he went abroad and spent some time at the University of Heidelberg.

With the exception of consultation work in nervous diseases, Dr. Van Gieson never entered private practice, but devoted practically his entire professional career to research work. Filled with the spirit of the born investigator, his interest embraced a wide field of scientific medical research, but during the greater part of his professional life, his chief efforts were directed toward the investigation of the structure, functions and diseases of the brain and nervous system.

For some years he held the position of instructor in these subjects at the College of Physicians and Surgeons, New York, where his ability and wide knowledge of his subject enabled him to give a course of unusual interest and value.

When the Pathological Institute was organized by the State of New York in 1896 for the study of mental and nervous diseases, Dr. Van Gieson was chosen as its first director, and he remained in that position for about seven years. He also held the office of State Commissioner in Lunacy from 1896 to 1901, and became widely recognized as an authority on mental diseases. It was largely due to Dr. Van Gieson's initiative that important steps were taken for the correction of many abuses, and for the establishment of better methods of investigation in connection with the insane.

Our present knowledge of sunstroke, its nature, and the changes it produces in the nerve cells, owes much to the investigations of Dr. Van Gieson. He is also very widely known for his research work on the pathology of the nervous system in many other conditions, and also for the very valuable staining methods which he has perfected. Very important studies of hydrophobia and of some of the recent processes of diagnosis in that disease have been made by Dr. Van Gieson. Of late years he has been deeply interested in the study of tuberculosis, and was eagerly seeking to establish new methods of treatment and diagnosis when his work was cut short by his last illness.

To those who were privileged to work with him, there was continual inspiration in the boundless enthusiasm and tireless purpose which would sometimes lead Dr. Van Gieson to spend whole nights in his studies. Yet, no one who had experienced it can forget the kind interest with which he was ever ready to turn from his own work to help the veriest tyro in the scientific field, and there must be many who will hold him in grateful remembrance for his generous kindness in addition to their respect for his scientific achievements.

Dr. Van Gieson's fame rests secure upon the basis of his many valuable contributions to science, and the Research Laboratory is honored in having counted upon its roll for the past eight years the name of one who will always be recognized as an able and original investigator.

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DIFFERENTIAL DIAGNOSIS AND TREATMENT OF EPIDEMIC CEREBROSPINAL MENINGITIS

Phebe L. DuBois

In 1906 the New York Board of Health supplied antimeningitis serum for use subcutaneously in cases of epidemic cerebrospinal meningitis, but it was found to be of slight avail and its preparation and use were discontinued. In July, 1910, after Jochmann, Flexner and Wassermann had proved its value when used intraspinously, the Rockefeller Institute gave the Health Department two horses immunized against the meningcoccus, and a generous supply of antimeningitis serum. Since that time the Health Department has prepared it and offered the services of a consultant to every physician reporting a case of meningitis not in a hospital. In that way we have seen 247 cases, at least half of which were reported as epidemic cerebrospinal meningitis and the rest either as meningitis, kind not designated, or as tuberculous meningitis.

Diagnosis

Table 1 shows what a large percentage of error there must be in the usual statistics of meningitis and how few cases of epidemic cerebrospinal meningitis there are in New York.

The cases of infantile paralysis that we have seen have been of the encephalitic rather than the myelitic type.

The streptococcus, pneumococcus and influenzal cases could not have been differentiated except by lumbar puncture, though the history of middle-ear disease, injury such as fracture of the skull, operation on the nose, etc., often made us suspicious.

In the cases of pneumonia, gastro-enteritis, hysteria, simple neurosis, acidosis, typhoid, osteomyelitis and serous meningitis, the rigidity of the neck, and in some cases a Kernig sign was probably what caused the physician to report the case as meningitis. In the case of Schönlein's purpura, one of the cases of typhus, and the eczema, erythema multiforme and measles cases, the eruption was largely responsible for the diagnosis of meningitis. Two of the cases of typhus had already been diagnosed and we were asked to do a lumbar puncture in order to relieve pressure. The mental condition seemed to have suggested meningitis in the cases of brain syphilis, endarteritis and miliary tuberculosis. The meningismus coming early, before there are any signs of pneumonia, is the most difficult of all these conditions to distinguish from true meningitis. Often by the time we reach the case the real diagnosis is apparent. In view of the mistakes made in diagnosis, I shall give a brief summary of the main points to be considered.

Cases of epidemic cerebrospinal meningitis and infantile paralysis usually give a history of a sudden onset as against a slower onset in tuberculous meningitis. The patient's mental condition is generally good in meningococcus meningitis unless it is a fulminating case. In tuberculous

TABLE 1

FINAL DIAGNOSIS OF CASES SEEN

Diagnosis.	No. of Cases	Condition Suggesting Meningitis
Epidemic cerebrospinal meningitis	48	
Tuberculous meningitis	75	
Infantile paralysis	37	
Streptococcus meningitis	9	
Pneumococcus meningitis	3	
Influenzal meningitis	2	
Pneumonia	19	Rigidity of neck and Kernig sign.
Gastro-enteritis	5	
Typhoid	3	
Hysteria	1	
Acidosis	1	
Osteomyelitis	1	
Simple neurosis	1	
Tetany	3	
Serous meningitis	2	
Schönlein's purpura	1	Eruption.
Eczema	1	
Erythema multiforme	1	
Measles	1	
Typhus	3	
Syphilis of brain	1	Mental condition.
Cerebral endarteritis	1	
Miliary tuberculosis	1	
Encephalitis	1	Palsy.
Peripheral neuritis	1	
Internal hydrocephalus	1	Bulging fontanelle.
Toxic conditions	1	Convulsions.
Tetanus	1	
Diagnosis uncertain	19	
Pericarditis	1	
Normal child	1	

meningitis there is often a history of irritability followed by increasing stupor from which it is often impossible to arouse the child. In infantile paralysis the patient is often apparently stuporous, but he can be roused and made to answer intelligently. Patients with epidemic cerebrospinal meningitis show marked rigidity of the neck; tuberculous patients are apt to have slight or moderate anteroposterior rigidity, but none laterally.

In infants the rigidity of epidemic cerebrospinal meningitis is so easily overcome as to make one often doubt its existence; but when the infant is turned on the side, the head is markedly retracted. We have found Brundinski's and MacEwen's signs much more useful than Kernig's.

Brundinski's consists in the flexion and eversion of the legs and arms when an attempt is made to flex the head on the chest, and the drawing up of one leg when the other is passively flexed. MacEwen's is a change from the normal in the percussion note over the lateral ventricles, due to increased intraventricular pressure.

The fever is higher in infantile paralysis and epidemic cerebrospinal meningitis than in tuberculous meningitis. In cerebrospinal meningitis it runs an irregular course. In infantile paralysis it usually stays up only a short time and falls by lysis. Paralyses are not common in meningococcic meningitis, are often present one day and gone the next in tuberculous meningitis and usually improve slowly in infantile paralysis. Convulsions occur in all three conditions. Projectile vomiting is not uncommon with tuberculous meningitis.

These are the general points of differentiation, but sometimes it is impossible to be sure clinically with which of the three conditions we are dealing. The surest means is by the examination of the spinal fluid, but this is not always certain. Epidemic cerebrospinal meningitis in almost all cases gives a cloudy fluid from which the infecting organism can be isolated. Both tuberculous meningitis and infantile paralysis give a clear fluid under increased pressure. If the tubercle bacilli can be found in the stained sediment after centrifugating the fluid, the diagnosis is easily made. Dr. Hemingway made a record in finding tubercle bacilli in cerebrospinal fluids at the Babies' Hospital, but there repeated punctures were done and sometimes a search of several hours was necessary. We rarely make more than one puncture, and seldom have more than an hour to spend searching. At the Babies' Hospital fluids were allowed to stand until a fibrin web formed. Ours are usually shaken a good bit before they reach the laboratory and we often have to centrifuge them at once. We usually trust to guinea-pig inoculation for the final diagnosis when we do not find the bacilli.

Table 2 shows the characteristics of spinal fluids.

Treatment

The treatment of epidemic meningitis may be divided into prophylactic, specific and general. The prophylaxis consists in quarantining cases and looking for carriers. Last spring we were called to see a six-months-old Greek baby. The mother had come over in the spring of 1911 at the time when there was an epidemic of meningococcic meningitis in Greece, and the baby was born six months later. A culture from the mother's nasopharynx showed abundant meningococci. She had never had meningitis and did not develop it. There was no way of being positive that the baby was infected by her for she may have acquired the meningococci from the baby; but it seems probable that she was the carrier.

Now, when we go on cases, we make cultures from the throats of those coming in contact with the patient. We have not yet had sufficient experience in ridding carriers of their meningococci to know what is the best means, but we hope to find out this winter. Dr. Sophian found that the injection of meningococcic vaccine was effective. Hachtel and Hay-

TABLE 2

CHARACTERISTICS OF SPINAL FLUIDS

	Pressure	Amount, c.c.	Appearance	Cytology	Bact.	Alb.	Glob.	Animal Inoc.
Normal.....	Normal.	5–10	Clear.	Very few cells.	Sterile.	±	—	Negative.
Meningismus.....	Increased.	10–100	Clear.	Very few cells.	Sterile.	±	—	Negative.
Infantile paralsyis.	Increased.	20–100	Clear; sometimes slight fibrin web.	Early polynucleosis; later lymphocytosis up to 95 per cent.; endothelial cells.	Sterile.	+-++	+-++	Negative or pneumonia.
Tuberculous meningitis.	Increased.	30–120	Clear; fibrin web.	Lymphocytosis up to 95 per cent.	Tubercle bacilli.	++-+++	++-+++	Tuberculosis in 4 weeks.
Epidemic cerebrospinal meningitis.	Increased.	5–120	Cloudy.	Polynucleosis up to 98 per cent.	Meningococcus.	++-++++	++-++++	
Meningitis due to other organisms.	Increased.	20–100	Cloudy.	Polynucleosis up to 98 per cent.	Infecting organisms.	++-++++	++-++++	

ward in Baltimore used a spray of antimeningitis serum effectively. In some experiments which Miss Bertha Warren and I made at the Research Laboratory last winter we found that of the usual disinfectants used in the throat argyrol in a strength of from 15 to 25 per cent. was the most effective in killing meningococci *in vitro*.

The specific treatment consists in the intraspinal injection of antimeningitis serum. We always inject the serum if we find a cloudy fluid on lumbar puncture, because if the disease is meningococcic meningitis the sooner the serum is injected the better, and it does no harm even if some other organism is the cause of the trouble. We always warm the serum and allow it to run slowly by gravity. The amount administered depends on the amount withdrawn; we usually inject serum a little less in amount up to 40 c. c. than the cerebrospinal fluid withdrawn. Using change in blood pressure as a guide to the dose, as suggested by Dr. Sophian, is probably a good thing, especially for those who have had little experience in administering the serum. I do not like to give a definite opinion about its value until I test it further. Most of our work is done in tenements with no one to help but the attending physician, so that its use is usually impracticable for us.

In an ordinarily severe case we do a lumbar puncture, draw off all the fluid we can and inject serum every day for four days. Further injections depend on whether the fluid is sterile or not, the patient's temperature, and his general condition. The rigidity of the neck is frequently late in disappearing, and if the patient is improving in every other way that does not disturb us. Dry taps occur now and then wthout disturbance of the communication between the meninges of the brain and cord. We had a case in Brooklyn in which after the first two or three times we repeatedly obtained no fluid; and yet 10 c. c. of serum ran in readily. The patient, a six-months-old infant, seemed moribund when first seen, but recovered completely. We have had a serum rash in but one case. For cases that threaten to become chronic we prepare autogenous vaccine. Whether or not it helps we shall not be able to say until we have tried it longer.

Patients who develop basic meningitis, in which the communication between the meninges of the brain and cord is cut off, have never recovered in our experience. We have sent several such into the hospitals, and brain puncture was done; but it has been of no avail. Dr. Sophian had the same experience in Texas during the epidemic last spring.

For general treatment we always recommend the administration of hexamethylenamin (urotropin) because experiment has shown that after its administration formaldehyd is found in the cerebrospinal fluid. We advise the use of sedatives when a patient is in much pain or very restless, in order that enough sleep may be obtained. The diet should not be too limited, for the duration of the disease may be prolonged. The conjunctivitis sometimes present is best treated with argyrol. The bladder must be watched, for many cases develop retention. Meningococci are sometimes found in the urine, and this must be remembered in ordering the hygiene of the sickroom.

Before the use of the serum the mortality averaged 75 per cent. Our mortality since using the serum has been about 50 per cent. Six cases developed basic meningitis and five cases were fulminating. During an epidemic basic and fulminating cases do not occur in such a large proportion. Several patients we did not see until late in the disease, and many were receiving poor care as far as nursing went. Dr. Sophian went to Texas for the New York Health Department during the epidemic last spring, and he had a mortality of 28 per cent. in the hospital. Dr. Flexner's analysis of the results in 1,300 cases agrees very well with this. The prognosis is better for children than for adults.

TWO CASES TREATED WITH ANTISTREPTOCOCCUS SERUM

MATTHIAS NICOLL, JR.

1. SEPTIC SCARLET FEVER

The patient was treated at the hospital of the Rockefeller Institute on the service of Dr. Rufus Cole, to whose courtesy I am indebted for permission to publish the clinical history as well as the account of the bacteriological tests made at the Institute.

Bessie Q., age 12, admitted March 26, 1913, on third day of disease. Very toxic and apathetic.

Throat: tonsils much swollen and ragged, the left on inner and posterior surface covered by dirty yellowish white necrotic looking pseudomembrane. Much dryish mucopurulent material in the nasal pharynx.

Left ear drum red.

Lymph nodes; anterior cervical greatly enlarged and firm.

Short high-pitched murmur at apex and along the left sternal margin.

Intense general scarlet eruption, many petechial points. Capillaries very brittle; vaginal discharge, no gonococci. 5,000 units diphtheria antitoxin given.

March 27. Patient irrational; throat culture shows streptococci, no diphtheria bacilli.

March 28. Patient continues irrational; throat very rotten; breath foul. Anterior cervical nodes very large and boggy. Heart rapid, sounds muffled, presystolic gallop rhythm. Patient given 160 c. c. antistreptococcus serum intravenously. The temperature fell 5° F. in two hours, beginning three hours after serum administration. See Chart I.

March 29. Patient rational; throat beginning to be less dry and clearing. Small amount of fluid in each knee joint.

March 30. Two-hourly application of antistreptococcus serum to throat.

March 31. Throat noticeably improved.

April 1. Few sibilant sounds at both bases; patient very apathetic; still some fluid in right knee joint. 30 c. c. of antistreptococcus serum given subcutaneously. Since last note steady improvement; desquamation general and active. For past four days there has been a trace of albumin in the urine. Only two or three granular casts have been seen, but many leucocytes.

Except for a rise of temperature which stayed above normal for several days and was probably due to the effect of the serum, the patient made an uneventful and perfect recovery.

BLOOD CULTURE A

Blood taken from vein of arm into two 100 c. c. broth flasks on March 26, 1912, third day of disease. About 5 c. c. blood in each flask.

Flask incubated. No change until March 28, 1912, when broth

showed blood purplish, with broth darkened and a precipitate suspended in it. Microscopic: Both flasks showed (March 28) groups, chains and isolated forms of small cocci, rather faintly stained.

Subcultures made on following media and incubated 12-18 hours.

(*a*) Sheep serum glucose agar slant:

Macroscopic: Pin-point, colorless, slightly raised, closely packed colonies with a few slightly larger, whitish colonies scattered in.

Microscopic: Both kinds show cocci in chains, neither resembling staphylococci. Some of the cocci are small, faintly stained and some medium sized and more deeply stained.

(*b*) Glucose agar stabs:

Macroscopic: No gas. Even, colorless growth along stabs.

Microscopic: Small cocci in long chains and a few deeper stained diplococci also in chains.

(*c*) Milk (Blue litmus):

Macroscopic: Turned very pink.

(*d*) Broth:

Macroscopic: Whitish granular sediment giving even cloud on shaking.

Microscopic: Long chains of rather faintly staining, small diplococci and a few diplococci of a little larger size, more deeply stained and with less tendency to form chains.

(*e*) Blood agar plate:

Macroscopic: Even distribution of minute, pin-point colonies with circles of hemolysis and of very small, pin-head sized, brownish colonies without hemolysis.

Microscopic of former: Medium sized cocci in pairs, masses, singly and in very short chains.

Microscopic of latter: Small, faintly staining cocci, mostly in short chains resembling the strain isolated from the throat. The larger was at first taken for an atypical form, hence not separated.

Gram positive.

Virulence: On March 29th mice were inoculated as follows with varying amounts of a 12-18 hour broth culture of March 28th, subcultured from one of the original flasks:

Mice injected at 11 a. m., March 29th.

0.01 c. c.

0.05 c. c. Lived several days at least.

0.10 c. c.

0.50 c. c. Found dead at 8:30 a. m., March 30th, *i. e.*, within 22 hours after inoculation.

1.00 c. c.

On April 3d, the following experiment was undertaken in order to test the protective power of the antistreptococcus serum used for the patient, when mice were inoculated with the streptococcus obtained from her blood.

Six mice were injected intraperitoneally at 12:50 p. m., each with 0.5 c. c. of the serum. At 4:00 p. m. five of these mice were inoculated intraperitoneally with varying amounts of a fresh culture of the blood streptococci as follows:

0.5 c. c. of broth culture. Mouse still alive April 8th.
1.0 c. c. of broth culture. Mouse still alive April 8th.
2.0 c. c. of broth culture. Mouse found dead at 7:30 a. m., April 5th.
3.0 c. c. of broth culture. Mouse found dead at 7:30 a. m., April 5th.
5.0 c. c. of broth culture. Mouse died 3:00 p. m., April 4th, 23 hours after inoculation.
0.0 c. c. (serum control) of broth culture, appeared normal for days.

Hence 0.5 c. c. of serum gave partial protection against ten times the minimum lethal dose of broth culture.

Blood Culture B

The positive culture was obtained on March 26th. 160 c. c. of the serum was given intravenously on March 28th. The second blood culture (B) was taken in the same way as the first on March 29th, with the patient wonderfully improved clinically. It was the sixth day of the disease. Two blood agar plates were made directly from the vein, putting about 1.5 c. c. of blood in each. Two broth flasks (100 c. c.) each received aboct 4-5 c. c. of blood. No growth appeared or could be demonstrated in any of these during the first ten days after the culture was made. Not studied further.

2. Post Partum Septic Pneumonia

The following case occurring in the practice of Dr. L. N. Lanehart of Hempstead. Dr. Theodore Janeway, consulting physician, to whom I am indebted for permission to publish the history, illustrates the apparently beneficial effects of large and repeated doses of antistreptococcus serum given intravenously. The serum used in this as well as in the first case was furnished by the Research Laboratory and came from a horse receiving many different strains of streptococci (killed, autolyzed and living).

Mrs. J. R. S., Age 35. Confined with third child, Dec. 25th. Puerperium normal until 9th day, then chill, temp. 101° F., foul lochia.

Jan. 4th (10th day). Chill repeated; 11th day temp. rose to 102° F. without chill; 12th day, acute pain in right back, which by the next day was severe on deep inspiration. Cough, bloody sputum and marked prostration. Breasts now dry.

Jan. 8th (14th day). Prostrated, cheeks flushed, tongue dry, slight jaundice. Pulse 120, very soft, small. Marked consolidation of right lower lobe. Otherwise negative. No evidence of femoral thrombosis. Leucocytes increased, Poly-morph. 80 per cent., Mononucl. 20 per cent.

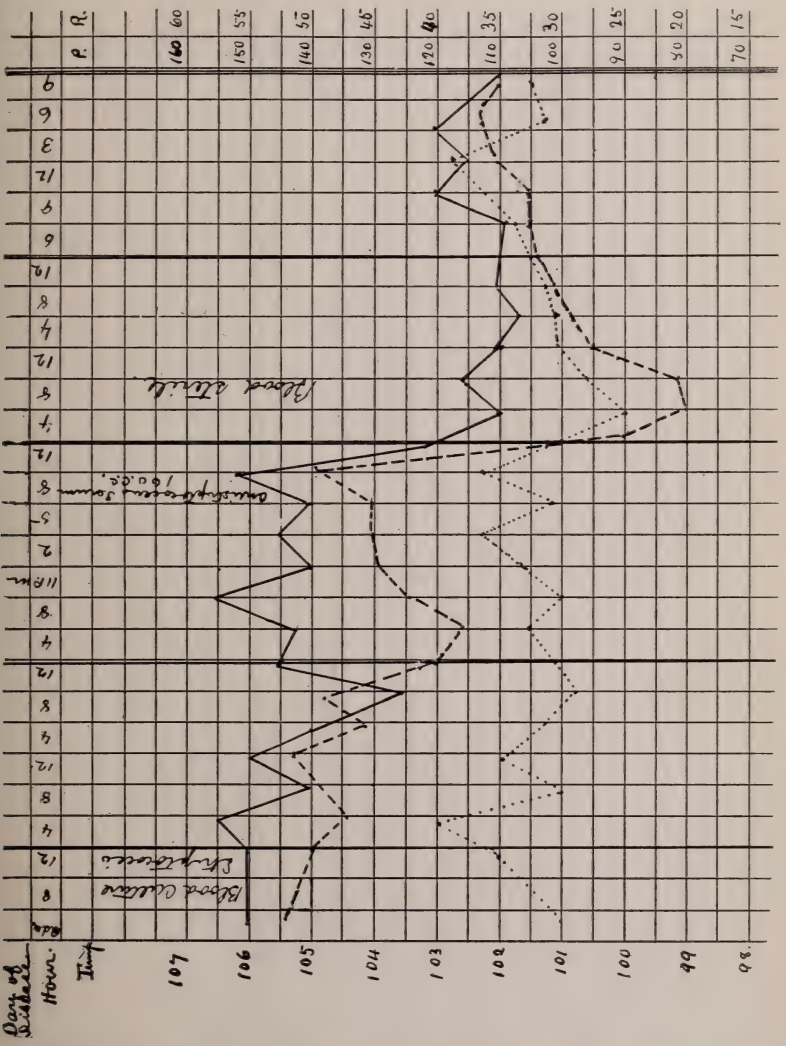


Chart No. I.

Diagnosis: Thrombophlebitic form of uterine sepsis with embolic pneumonia.

Jan. 9th, 10th and 11th. 100 c. c. antistreptococcus serum given intravenously with rapid improvement.

The temperature fell to normal on Jan. 13th and remained so until Jan. 17th, when it gradually rose and remained high for four days, coincident with the occurrence of serum sickness.

Jan. 15th. Six days after first injection, urticaria.

Jan. 16th. Spreading.

Jan. 17th. Pain and stiffness in joints, spreading rapidly with rising fever on 18th.

Jan. 19th. Rash beginning to fade, joint pains severe. Blotchy erythema with wheals on face, arms and legs. Diffuse erythema on chest. All joints stiff and painful on movement. Spleen just palpable. Slight oedema of legs, feet and hands. Heart normal. Blood pres. 95 syst., 65 diast., rate 100. Signs of resolving pneumonia over right lower lobe. 8 minims adrenalin chloride 1-1000 injected subcutaneously. Marked subsidence of erythema in 5 minutes, striking in 10 minutes, with relief of lameness of fingers, wrists and elbows, but persistence of pain in leg joints.

Diagnosis: Serum disease, severe type.

After this adrenalin given in same way every 4 hours with relief.

25th. Eruption disappeared, 7 days after its appearance. Joint pains on 23d day.

Uninterrupted recovery from both sepsis, pneumonia and serum disease.

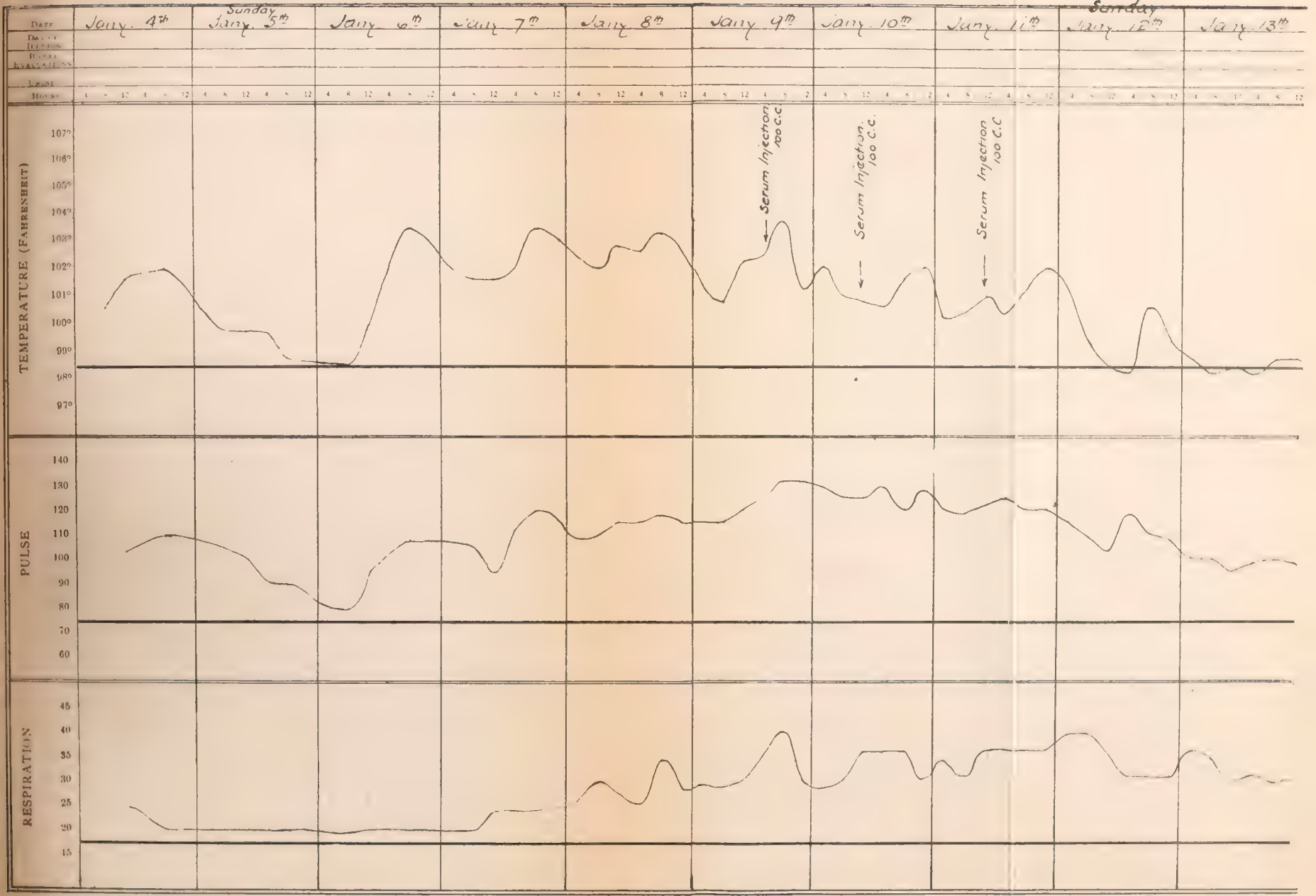

Date
Jany. 4th
Sunday
Jany. 5th
Jany. 6th
Jany. 7th
Jany. 8th
Jany. 9th
Jany. 10th
Jany. 11th
Sunday
Jany. 12th
Jany. 13th
Hours
4 8 12 4 8 12
TEMPERATURE (FAHRENHEIT)
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
Serum Injection 100 C.C.
Serum Injection 100 C.C.
Serum Injection 100 C.C.
PULSE
140
130
120
110
100
90
80
70
60
RESPIRATION
45
40
35
30
25
20
15

Chart No. II

THE DOSAGE AND METHODS OF ADMINISTERING DIPHTHERIA ANTITOXIN: AN EXPERIMENTAL AND CLINICAL STUDY

William H. Park and George P. Biggs

Twenty years have elapsed since von Behring published the evidence that diphtheria antitoxin had not only demonstrated its antitoxic value in animals, but also in man. Since then hundreds of thousands of patients sick with diphtheria have been treated with antitoxin. Nevertheless, it remains as true to-day as at any previous time that capable physicians of great experience differ radically as to the best way to administer the serum and as to the amount required. If we study the publications of the past five years, we find very great divergence of opinion. McCollum from his experience in the Boston City Hospital advised in mild cases for the first injection 4,000 units and in severe cases 10,000. He urged the repetition of this dose every four to six hours until exfoliation of the membrane occurred. This means from 12,000 to 80,000 units in each case of diphtheria. Royer, after an extensive experience in the treatment of diphtheria in Philadelphia, advised the same initial dose, but repeated in intervals of twelve to twenty-four hours instead of four to six hours. This gives a total in each case of from 6,000 to 20,000 units. Howland, with his experience in the Willard Parker Hospital in New York, advised the same initial dose, but believes one injection sufficient. The total here amounts to 4,000 to 10,000. He is the only one of large experience so far as I know who advocates the single injection in severe cases. Some of the foreign authorities believe smaller doses than those above advised are sufficient. Thus Baginsky considers 3,000 units enough for the first injection in quite severe cases.

The influence on dosage which the size of the patient should have is judged differently by different trained observers. Baginsky advises that one-third more be given to adults than to small children; Hutinel of Paris gives double the amount; Ker of Edinburgh gives the same to all ages, with the exception of infants under one year, who receives one-third less. Koplik considers size an important matter and gives children under one year for a first injection 1,500 to 3,000 units, while those over five years 5,000 to 10,000 units. Those between these ages should receive amounts between these figures. My opinion upon this point is that in mild or moderate cases infants under 30 pounds should receive one-half less than older children weighing 60 pounds or more, while in very severe cases persons should receive antitoxin in proportion to their weight. There can be, of course, no question that the antitoxin content of the blood of cases receiving the same amount of antitoxin is roughly proportioned to their weight as that controls the amount of blood and that in turn the dilution of the antitoxin. The greater element of danger in infants causes most clinicians to give them a greater amount than their weight calls for.

The above opinions were founded largely on clinical experience. Some of the best clinical observers have stated their belief that laboratory experiments on animals or healthy men have been valueless; thus in Osler's Modern Medicine McCollum states, "In serum therapy the dose cannot be fixed by definite rules. Laboratory experiments do not give any assistance because there is no known method of estimating the quantity of toxins." He also states that intravenous injections are not necessary since the antitoxin is rapidly absorbed from the subcutaneous tissues. Royer states that the first injection of antitoxin is frequently utilized in caring for the toxin in the circulating fluids and that the early disappearance of the antitoxin was because of this neutralization and required the additional injection.

The results of our investigation, which combine laboratory experiments with clinical observation of cases, prove that it has been a mistake for clinicians to cling so exclusively to bedside observations.

The Rate of Absorption from the Subcutaneous Tissues

Scattered observations have been made upon this subject from time to time since the beginning of the therapeutic use of diphtheria antitoxin. Von Behring made observations on the absorption of antitoxin in a goat in 1897 and Bullock in 1899. Ransom[1] in 1901 showed that antitoxin injected into the subcutaneous tissues is removed by the lymphatics. J. H. Smith[2] tested the absorption in man in 1907. He injected 9,000 units subcutaneously in a man and from time to time took bleedings and tested the antitoxin strength. The findings are shown in Table 1.

TABLE 1

One c. c. of the patient's serum contained:

Before injection:	no demonstrable antitoxin.
5 hours after injection:	0.1 units antitoxin.
14 hours after injection:	0.225 units antitoxin.
32 hours after injection:	0.68 units antitoxin.
44 hours after injection:	1.0 units antitoxin.
3 days after injection:	1.3 units antitoxin.
4 days after injection:	1.3 units antitoxin.
6 days after injection:	0.68 units antitoxin.
13 days after injection:	0.17 units antitoxin.
15 days after injection:	0.14 units antitoxin.
20 days after injection:	0.08 units antitoxin.
27 days after injection:	No demonstrable antitoxin.

The results of these investigations indicated that the absorption of antitoxin from the subcutaneous tissues was a slow process and that it went on continuously at almost the same rate for 24 to 36 hours, and then more slowly for another 36 hours. It is noted that the blood content reached its maximum antitoxic strength between the third and fourth days, and that although it then decreased slowly, the amount present was still considerable at the end of a week. Smith concludes that because of

[1]Berlin. klin. Woch., 1901, XXXVIII (Part 1).
[2]Journal of Hygiene, 1907, VII, 205.

this slow absorption, intravenous injections are advisable in bad cases so as to get the full effect immediately. We have repeated the absorption experiments in four healthy men with substantially the same results (see this volume, page 269).

Original Investigations

The Antitoxic Content of the Blood in Persons Suffering from Diphtheria at Different Intervals of Time After a Subcutaneous Injection

A number of persons suffering from diphtheria and differing in age and severity were given a single subcutaneous injection of 10,000 units of antitoxin. They were then bled every six to twelve hours and the antitoxin content of the different bleedings noted. The results are plotted in Chart 1. The antitoxin content of the blood in these cases might be expected to differ from that in normal men for two reasons. The toxin in the blood and tissue fluids in some of the cases might be sufficient to neutralize the antitoxin to an appreciable degree, while on the other hand after the third day a development of antitoxin might occur in the tissues of the sick which would add to that in the blood due to the injection. The first possibility can be excluded, for in no case was the amount of free toxin sufficient to neutralize enough antitoxin to appreciably lower the percentage in the blood. Tests taken previous to injection showed that none of the cases had more than a trace of free toxin in the blood. The charts show that in fact the blood of two had already appreciable quantities of antitoxin. The tests showed that the antitoxin content of the blood after the injection at first increased gradually as in healthy men. The absorption was most rapid during the first twelve hours, a little less during the second twelve hours. From this time the cases differed, according as to whether antitoxin formation took place in the body or not. In one case, a child of fifty pounds, the body cells so actively produced diphtheria antitoxin that one c. cm. of the blood on the fifth day contained 60 units. In some of the other cases only a very little or no antitoxin was produced. In none of these cases did the antitoxin content of the blood decrease until after the third day and in all it remained in large amount until at least the seventh day. The relation of the antitoxin content of the blood to the weight of treated cases is a point of interest. A curious fact developed in the different persons that, while in a very general way the antitoxic content of the blood after an injection of antitoxin was inversely proportional to the weight of the person injected, a wide difference occurred in individual cases, some having more than twice that of others of the same weight. This is seen most clearly in the table showing the absorption curves in normal men (page 269). A large number of diphtheria cases where but one or two bleedings were taken showed the same discrepancy. This is apparently due not only to proportional differences between the weight of individuals and their quantity of blood but also to some destruction or elimination of the antitoxin.

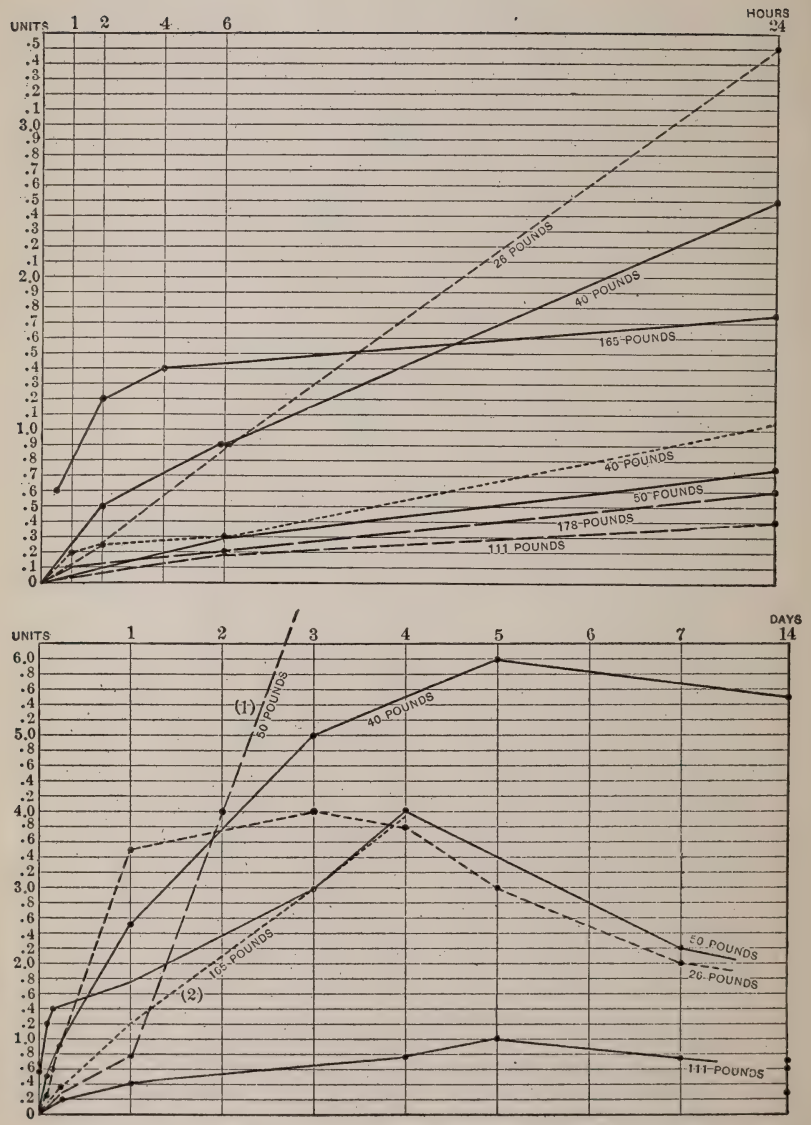


CHART I.—Showing the extent and rapidity of absorption of 10,000 units of antitoxin given subcutaneously. Each line represents the antitoxin content of 1 c. c. of blood at different intervals of time.

An Immediate Injection of Sufficient Amount Adequate for the Treatment of a Case Important. Subsequent Injections Unnecessary

A study of the charts showing the actual content of antitoxin in the blood in cases of diphtheria at different intervals after an injection of antitoxin emphasizes strongly the truth taught by the tests in a normal man by J. H. Smith—that antitoxin is absorbed very slowly and is retained for some days. The actual formation of antitoxin after forty-eight hours in persons suffering from diphtheria adds still further to the content of the blood and eliminates completely the necessity of giving injections beyond the first adequate one. Animal experiments illustrating the superior results following a single subcutaneous injection and the same amount given in two or three divided doses at intervals of six hours makes the importance of the single injection clear.

Content of Antitoxin in Blood of Goats Injected With 10,000 Units *

Hours After Injection	Units in 1 C. Cm. Receiving Single Injection	Receiving same Amount Divided in Three Portions	Receiving same Amount Divided in Two Portions
3	1	.33	.5
6	2	.66	1.0
12	5	2.3	3.5
24	9	7.0	8.0
48	17	15.5	16.0

The importance of giving a sufficiently large initial dose and the inadequacy of trying to repair the mistake by subsequent injections is brought out clearly in the following experiment, in which a dose sufficient to save life when given as one injection is ineffective when divided.

Rabbit A—This received 10 fatal doses of toxin intravenously and 20 minutes later 100 units of antitoxin. Total antitoxin 100 units. It died.

Rabbit B—Same details as A, except 200 units of antitoxin were given. Total antitoxin given 200 units. It lived.

Rabbit C—This rabbit received at 20 and 40 minutes 100 units of antitoxin and at 60 and 80 minutes 150 units. Total antitoxin given 500 units. It died.

It is important to note that in these animals an amount sufficient to save when given in one injection was insufficient when divided.

Some who have observed cases carefully at the bedside, while they have to accept the fact of this continuous absorption during the first forty-eight hours, object to restricting themselves to a single dose, because they have seen the local conditions decidedly improve immediately after a second or third injection. Careful consideration of the facts previously stated will overcome this objection. The apparent quick response to the second and third injections of antitoxin is not real, for it happens just

* These figures are estimated from tests on several different animals. The weights are equalized at 30 pounds.

as certainly if later injections are withheld. No amount of antitoxin will cause an appreciable change in local conditions within a period of six hours, and the exfoliation of membrane requires much longer. It is impossible for enough of the later injected antitoxin to be absorbed within a few hours' time to add appreciably to that absorbed from the first injection.

Clinical Observations

Since January, 1911, the greater proportion of the cases at the Willard Parker Hospital have received but one subcutaneous injection. At first only a small proportion of the severe cases received but one dose, but as these did just as well as those receiving two or more, the attending physicians gradually reduced the number in which they ordered repeated injections. This is well shown in the record of the cases for the year 1912, when only 5.5 per cent. received more than one injection. The mortality in the 1,638 cases was only 11.77 per cent. This is the lowest rate in the history of the hospital.

Number of Injections and Dosage for Year 1912

Number of cases receiving one dose only	1,552
Number of cases receiving two doses	84
Number of cases receiving more than two doses	2
Those receiving less than 5,000 units	53
Those receiving 5,000 units	461
Those receiving between 5,000 and 10,000 units	10
Those receiving 10,000 units	946
Those receiving 15,000 units	63
Those receiving 20,000 units	41
Those receiving over 20,000 units	3

One factor in influencing the physicians to be satisfied with one injection was the estimation of the antitoxic content of the blood just before giving the second dose. As antitoxin in large quantity was shown to be invariably present, the attending physicians, who were at first opposed to the single dose method, soon became convinced and when later cases receiving but one injection pursued the same course as the earlier ones in which a second dose was given they showed less tendency to revert to the older method. As has already been stated, the mortality was lower during 1912 than during any previous year. This, in so far as it was due to the change of antitoxin treatment, was, of course, due to the fact of giving a sufficiently large dose at the first possible moment and not to the general elimination of the second and third doses. While these are unnecessary they are not harmful.

The Influence of an Early Injection Upon the Success of Treatment

Numerous experimental studies have demonstrated that a small amount of antitoxin will save when given before or shortly after the injection of toxin and that for each minute that elapses larger and larger amounts are required until finally no amount will save. The following

experiments in which intravenous injections of toxin were followed by intravenous injections of antitoxin are so striking that they are given, even though they are similar in some respects to the published results of others. The experiment already recorded indicates clearly that a dose sufficient when given as a single dose is insufficient when divided into several doses, even though the total amount is increased.

A number of rabbits were given intravenously ten fatal doses of diphtheria toxin. At different intervals of time antitoxin was given, the following amounts were required to save at the different intervals.

Amount Sufficient to Save Life

Given after 10 minutes.....	5 units.
Given after 20 minutes.....	200 units.
Given after 30 minutes.....	2,000 units.
Given after 45 minutes.....	4,000 units.

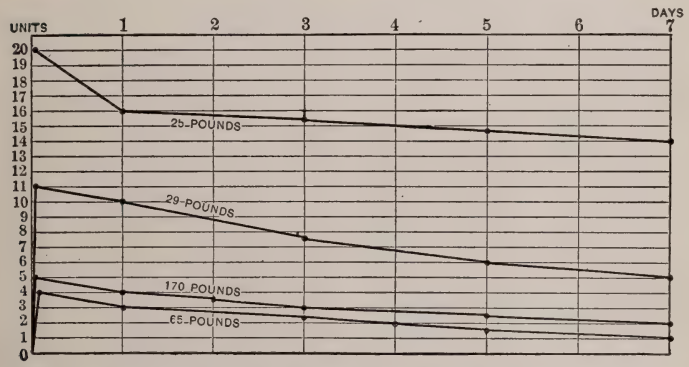


CHART II.—The antitoxic power of human blood after an intravenous injection of 10,000 antitoxic units.

Amount Sufficient to Save Life

Given after 60 minutes.....	5,000 units.
Given after 90 minutes.....	No amount.

These results emphasize the need of haste in giving antitoxin in serious cases. Fortunately in the ordinary case the diphtheria poison has not reached the blood current in any large amount at the time the patient is seen. The severe septic cases have, on the other hand, absorbed a great deal of toxin when we reach them. Here every minute's delay is of importance. In the moderate cases delay in giving antitoxin allows the local lesion to advance, but unless this becomes very extensive the only harm caused is delay in recovery. In the severe cases the intravenous injection of antitoxin is always indicated as no time is lost due to slow absorption from the subcutaneous tissues. The curves on Chart II., when compared with those on Chart I., show clearly the advantage of intravenous injections. In the mild and moderate cases the intramuscular or subcutaneous methods suffice. The rapidity of absorption from the

intramuscular tissues has been demonstrated in animal experiments to be about twice that from the subcutaneous tissues. Its administration in actual cases of diphtheria in our investigation did not usually show such an increased rapidity of absorption. This is probably because the serum did not remain within the muscle sheath. The following dosage of antitoxin is now used by us:

ANTITOXIN DOSAGE

	Units in Cases			
	Mild	Moderate	Severe	Very Severe
Infants under 1 year....	2,000	3,000	10,000	10,000
Children 1–5 years.....	3,000	5,000	10,000	10,000
Children 5–9 years.....	4,000	5,000	10,000	15,000
Persons over 10 years...	5,000	10,000	10,000	20,000

These doses were selected after considering both the relative degree of danger at the different ages and the importance of size upon the dilution of the antitoxin. The antitoxin should be given intravenously in the very severe cases. In all others the subcutaneous or intramuscular methods suffice to save life, but do not give as quick results.

Much smaller injections will suffice to save life in the majority of cases, but the larger doses advised will produce quicker local and constitutional effects and will in the more severe cases undoubtedly save some lives which would otherwise be lost.

It is conceivable that even larger doses might save an occasional life, but such a result is certainly rarely to be hoped for. The giving of antitoxin intravenously adds many times to its unit effectiveness. Every 1,000 units given into the circulation is worth at least 4,000 given subcutaneously. When only small amounts are available the antitoxin should be given intravenously.

SERUM SENSITIZATION AS RELATED TO DOSAGE OF ANTITOXIN IN MAN AND ANIMALS

William H. Park, Lemuel W. Famulener and Edwin J. Banzhaf

The results of a series of tests carried out by Lewis* showed that two units of antitoxin contained in 0.05 c. cm. of horse serum given one hour previously protected rabbits which received about ten fatal doses of diphtheria toxin. Both injections were given intravenously. When rabbits were sensitized by a previous injection of either normal or antitoxic horse serum, several times as much antitoxin was required. The amounts were not graded sufficiently to state the results more accurately.

Lewis recognizes that the experiments are limited in number and suggests that the curve of absorption of antitoxin in man as developed by J. Henderson Smith** be elaborated to cover the case of human beings who have received previous injections of normal or antitoxic horse serum. During the course of our studies upon the influence of protein concentration upon the absorption of antitoxin,*** we had an opportunity to make observations upon men which have a direct bearing upon this question. Also, further tests of like nature were carried out on goats. The results obtained from our tests have been plotted on charts for ease of comparison.

Experiments Upon Men

The data pertaining to the men are briefly submitted, as follows: S——— received on Feb. 11, 1913, an initial injection subcutaneously of 10,000 units diphtheria antitoxin in 4.5 c. cm. volume of a preparation containing 17.2 per cent. of pseudoglobulin. Two months later he received a second injection of a preparation containing the same number of antitoxic units and amount of pseudoglobulin, but diluted to 9.0 c. cm. volume. This was followed by a moderate local reaction. F——— received subcutaneously on Feb. 6, 1913, an initial injection of 10,000 units of diphtheria antitoxin in 9.0 c. cm. volume of a preparation containing 8.6 per cent. of pseudoglobulin. His second injection was given two months later. It contained the same number of units of antitoxin and the same amount of pseudoglobulin, but in a volume of only 4.5 c. cm. The local reaction was moderate in amount.

The above details are given for purposes of accuracy. The protein concentration and the volume of fluid are believed by us to have practically no appreciable effect. The results of these tests are represented graphically in Chart I, upon 100 lbs. basis.

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* Jour. Exper. Med., 1912, XVI, 216.
** Jour. Hyg., 1907, VII, 205.
*** Jour. Inf. Dis., 1914, XIV.

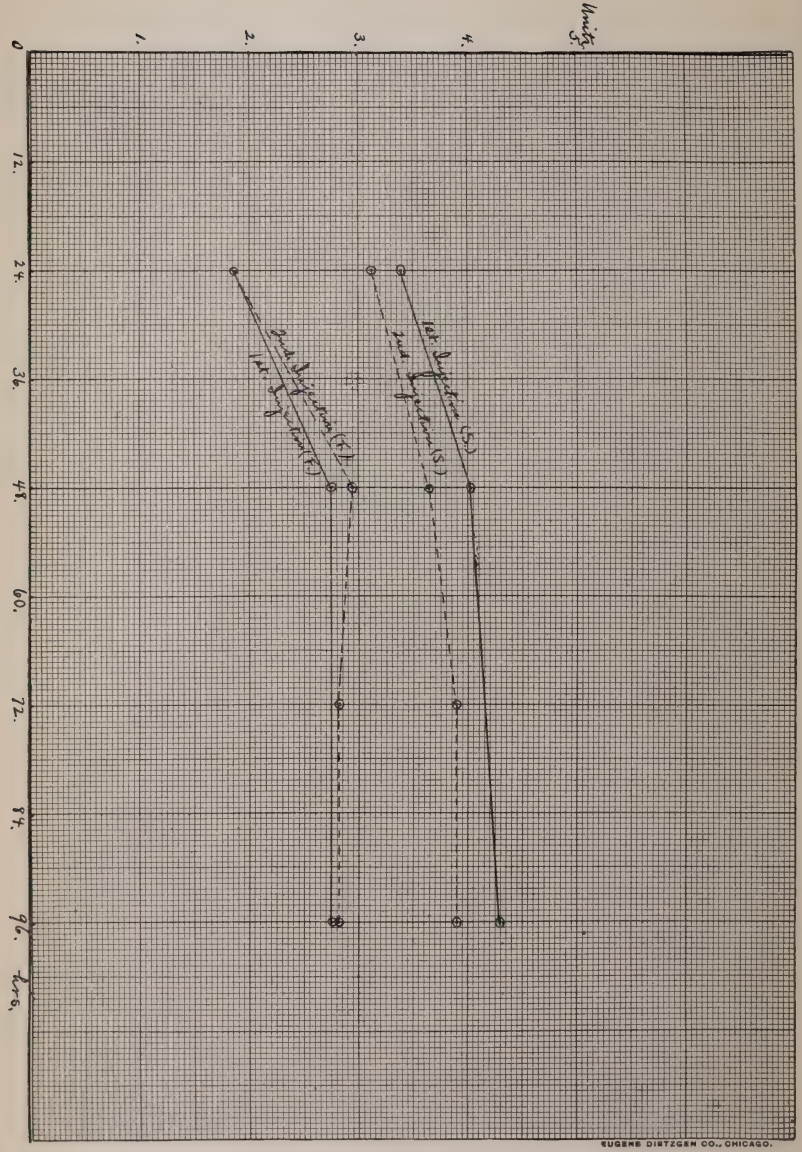


CHART I.—Comparative tests in man.

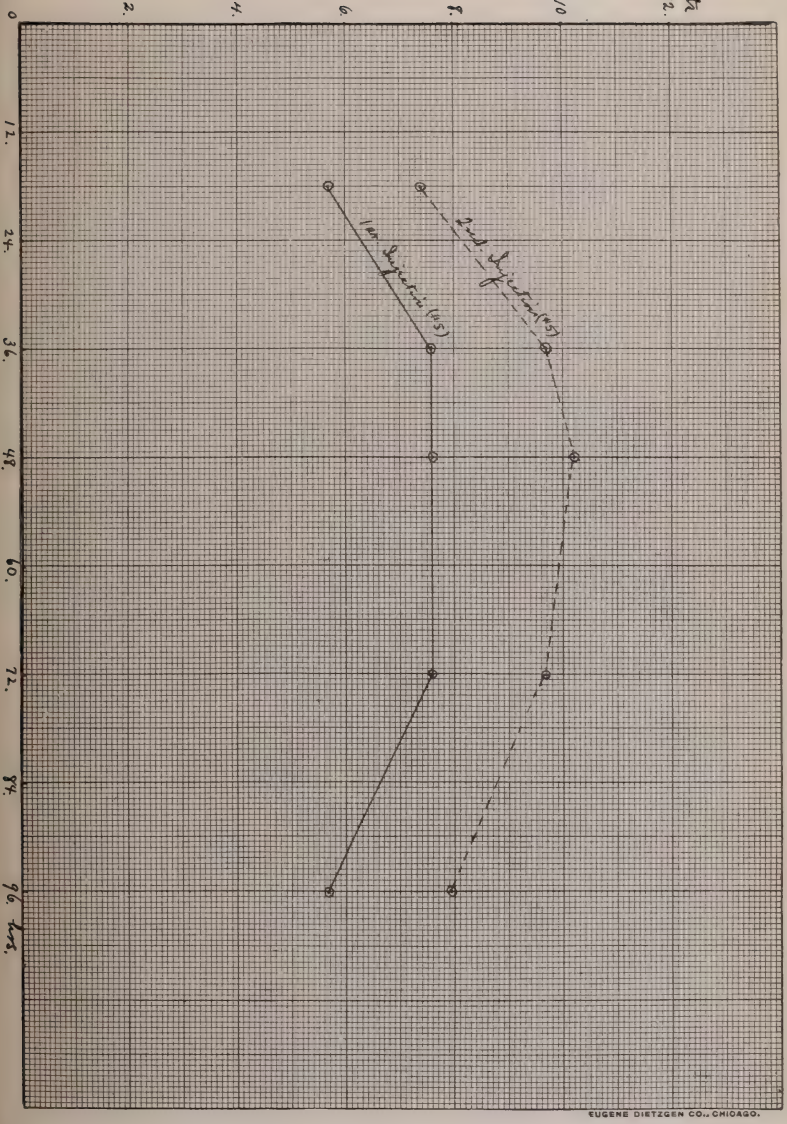


CHART II.—Absorption curves of first and second injections of antitoxin in Goat No. 5.

Experiments Upon Goats

Goat No. 5 received the first injection subcutaneously on Oct. 17, 1912, consisting of 10,000 units of diphtheria antitoxin in a volume of 10 c. cm. fluid containing 29.2 per cent. of pseudoglobulin. Its weight was 47.5 lbs. Slightly over seven months later the same animal received an injection of an antitoxic preparation corresponding in every particular with the first injection. At this time its weight had increased to 57 lbs. Chart II shows the results of both tests in curves with unit value per c. cm. of serum (goat), based upon 50 lbs. weight.

Goat No. 25 (weight 47.5 lbs.) was given subcutaneously a sensitizing dose of 10 c. cm. normal horse serum on March 19, 1913. On May 21, 1913, the animal was given a second injection subcutaneously of a diphtheria antitoxin preparation containing 10,000 units in 2.7 c. cm. volume with 29.2 per cent. of pseudoglobulin. For controls two animals (weights 54 lbs., 46.5 lbs.) were taken which had received primary injections of an antitoxic preparation which corresponded in unit strength, volume and protein concentration with that given in No. 25. The unit values of the serum samples from each animal were brought to the 50 lbs. basis, and in case of the two controls the average taken, then comparative curves were plotted as shown in Chart III.

Summary and Conclusions

The results obtained in two men and in two goats showed no appreciable difference in the absorption curves of antitoxin before and after sensitization. The variations that occurred in the cases appeared to be due to the inherent individual characteristics of the persons and animals injected and not to the sensitization.

We conclude that the large amounts of antitoxin injected in the treatment of diphtheria are neither bound nor destroyed appreciably by any globulin antibodies present in the blood of those previously injected. The same quantity of antitoxin is therefore indicated in the treatment of diphtheria whether the case has or has not received a previous injection of horse serum or globulins.

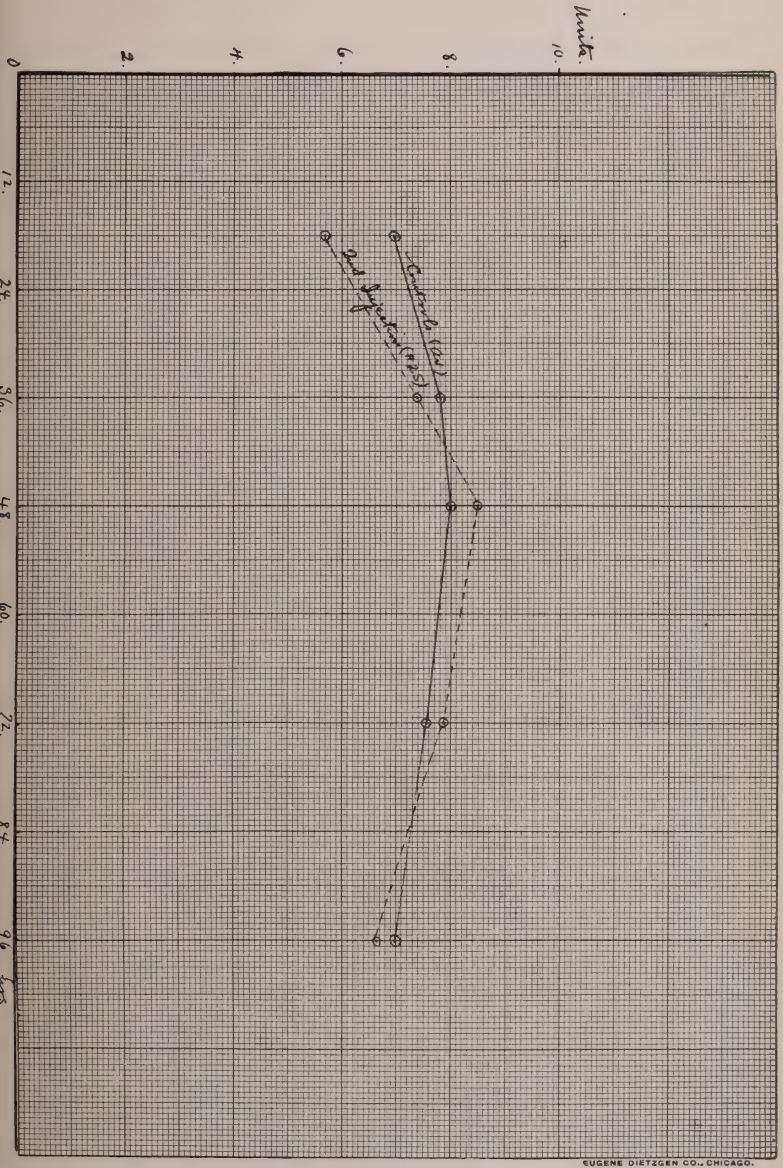


CHART III.—Average antitoxin content in two goats receiving first injections, and of one (No. 25) receiving a second injection.

TWENTY-THREE CASES OF PNEUMONIA TREATED WITH ANTIPNEUMOCOCCUS SERUM

WILLIAM R. WILLIAMS, M.D., ATTENDING PHYSICIAN, NEW YORK HOSPITAL

Through the co-operation of the Research Laboratory I was able to give a series of patients suffering from lobar pneumonia an antipneumococcus serum, and although the results were somewhat inconclusive, it seems desirable to publish the results.

The series includes practically all of the pneumonia cases admitted to the service of the Second Medical Division of New York Hospital during the period of the test. This division receives all medical cases admitted to the hospital on the even days of the month, while those admitted on the odd days go to the First Division. Although this method of assignment does not give a strict alternation of patients, yet it seemed justifiable to use the patients on the First Division as controls, in as much as none of them received any serum.

The serum was taken from a horse immunized against many strains of pneumococci.* Usually it was injected directly into a vein of the arm at a temperature about that of the patient. This rule was departed from and the serum was given subcutaneously in the case of two children where it was difficult to enter the vein with the needle, and also where second and later doses were given to four adult patients who had received the initial dose intravenously. The first doses for adults in the earlier cases was 100 c. c. This was increased later until, in one instance an initial dose of 300 c. c. was given. Many of the patients received more than one dose, so that the total amount of serum that an individual received ranged from 50 c. c. to 750 c. c. This maximal quantity was given in six doses spread over a period of five days.

A complete demonstration of the nature of the infecting organism was not essayed. Blood cultures, however, were made in eleven cases. Only one of them (Case 11) showed the pneumococcus, the others being negative. In Case 6 pneumococci were cultivated from pus from the pleural cavity. Cultures were made from the sputum in several instances.* The diagnosis was based on the clinical evidence of pneumonia, special weight being given to the presence of rusty sputum and the physical signs of a definite consolidation of the lung. In every case the subsequent course of the illness confirmed the diagnosis of lobar pneumonia and strengthened the probability of the existence of pneumococcus infection.

The chief points of note in these cases are given in a very condensed form in the accompanying table (Table 1), together with the details of the dosage of the serum. The time of onset of pneumonia is not uncommonly indefinite. Many patients give a history of an indefinite illness lasting for several days, during which time they may not have

* See note following.

been confined to bed continuously. This may explain the duration of some of the more protracted cases of this series. Most of the patients, however gave symptoms that fixed the beginning of the pneumonia with a reasonable degree of accuracy.

A review of the data of the table shows that the series included twenty-three cases with sixteen recoveries and seven deaths, a mortality of 30.5 per cent. Of the seven patients who died, three were alcoholics, one of whom had suffered from two previous attacks of pneumonia, and all were very actively delirious. A fourth death was that of a baby (Case 4) who had in addition to pneumonia multiple abscesses of the scalp, double purulent otitis media and empyaema followed by pneumothorax. The remaining three deaths seemed to have been due to the frank intensity of the pneumonia.

Of the sixteen patients who recovered, eight defervesced by crisis and eight by lysis. The crisis occurred on the following days: 4th, 5th to 6th, 6th, 7th, 9th, 11th, 12th and on the 18th to 19th. The onset of the lysis in the other cases that recovered occurred on the following days: 6th, 7th (3 cases), 8th, 10th, and on the 13th day. In the remaining cases the onset of the lysis was scarcely definable because the fall of temperature was so gradual. Five patients had complications. In one instance this was serous pleurisy (Case 5). In Cases 6 and 19 empyaema developed. In Case 4 the signs and symptoms were those of empyaema, but an exploratory puncture failed to find the pus. On the next day, however, there was evidence of pneumothorax, and on operation considerable pus was found. Two days later the child died with oedema of the lungs. She was extremely ill from the time of admission and had in addition to the pleural complications, double otitis media for which the drum membranes were incised, and also many abscesses of the scalp. Otitis was detected in Case 18 also. The series offered an opportunity to observe the effects of the administration of large and frequent doses of horse serum that had not been refined in any manner and that contained no preservative except a trace of chloroform.* Usually no immediate effects could be detected and the injection of the serum was tolerated surprisingly well. In five patients, however, symptoms appeared that should be mentioned more in detail.

Case 1, a boy of twelve years, received 50 c. c. of serum intravenously on the fourth day of his illness. A few minutes later he had a chill and his temperature rose from 104 F. to 107.2. This was followed by a startling drop, and within six hours of the injection his temperature was 97.6 F. The fever did not reappear, and convalescence was prompt and complete. I am inclined to regard this crisis as independent of the treatment, but at the time the sequence of events was very impressive.

Case 8 was a negro of thirty years, a rather heavy drinker and a driver of horses. When 80 c. c. of serum had run into the vein he suddenly developed severe dyspnoea, a very rapid and weak heart action, profuse sweating and oedema of the lungs. The serum was at once

* See note following.

stopped. Within a few minutes the symptoms began to abate and within half an hour the lungs were nearly clear and his condition was satisfactory. The subsequent course of the pneumonia was typical and but moderately severe. Six days later, on the ninth day of the disease, he defervesced by crisis. Convalescence was interrupted only by symptoms of serum sickness.

Case 20 was a boy of thirteen. During the introduction of the serum he complained of headache, pain in his abdomen and later in his legs. His pulse was accelerating and the serum was stopped, but not until he had received 200 c. c. Two hours later a profuse urticarial rash appeared on his abdomen and lasted a few hours. He had no late symptoms of serum-sickness.

Case 23 was a man of thirty-three, a heavy drinker. When 150 c. c. of serum had been given, he was nauseated and vomited. These symptoms ceased in about five minutes and 50 c. c. more of serum was given. At this time his pulse became rapid and he broke out in a profuse sweat The serum was stopped and in a few minutes he was comfortable. He likewise had no late serum symptoms.

The effect of the serum on the leucocytes was determined by frequent blood counts before and after the injections. No constant effects on the white cells could be made out. The results of some of these counts are detailed in Table 2.

Late symptoms due to the serum were noticed in eight patients. In consideration of the large doses of unrefined horse serum this is not a surprisingly large number. The symptoms were not usually severe.

During the time of this experiment the First Medical Division of New York Hospital treated fifteen cases of pneumonia with six deaths, a mortality of 40 per cent., as compared with 30.5 per cent. for the serum cases. Of their ten cases that recovered, six or 60 per cent. defervesced by crisis. In the serum cases 50 per cent. of the patients who recovered had crisis. Only two of the control cases had complications, a percentage of 13.3. In the serum cases five patients, 22 per cent., had complicating inflammations. It does not seem that these were due to the use of the serum but the number of patients with inflammations of the pleura (four cases, 17.4 per cent.) is uncomfortably large.

A study of the results of this treatment leaves one doubtful of the influence of the serum, in modifying favorably the course of the pneumonia.

The prosecution of this experimental study necessitated much extra work in the wards and laboratory. This was performed by the members of my house staff, Drs. Howard, Kutil, Alling and Wildman. I wish to thank them for their enthusiastic willingness which made these observations possible.

NOTES ON SERUM, CULTURES, ETC.

CHARLES KRUMWIEDE, JR., M.D.

The serum was prepared by injecting a horse with pneumococci from varied sources. These cultures were all typical inulin fermenting types. They were isolated from the mouth in cases of "Riggs Disease," from the sputum in cases of lobar pneumonia, from empyaema, the meninges and blood of septic infections following the exanthemata. The cultures used varied from time to time. As fresh cultures were isolated the older cultures were dropped. No attempt was made to raise the virulence of these cultures by passage through rabbits or mice. Although this may influence the protective value of the serum for these animals it is open to question what influence such adaptation to a new host has on the curative value in man. The cultivation on artificial media undoubtedly changes the pneumococci but how far this influences their ability to stimulate curative substances in the horse is unknown.

The injections at first were cultures in broth to which was added horse blood. Later the cultures were grown on agar and washed off with water. At first the increasing doses were of cultures which had been heated to 55 degrees C. for one hour. When the dose had reached the maximum decided upon, live cultures alternating with autolysate were used. The autolysate was prepared by allowing distilled water suspensions of the cocci to stand in the ice-chest for one week. The live cultures, or the autolysate, were added to the heated vaccine, in increasing amounts, until only the unheated culture or autolysate was given.

The injections were given once a week subcutaneously. The maximum dose equalled the growth on agar in ten-pint Blake bottles. The horse was receiving the total amount of live and autolysed cocci at the time it was supplied to Dr. Williams. The bleedings were made every two weeks. Every effort was made to supply the fresh serum to avoid any possibility of deterioration. After the serum was drawn from the clot several cubic centimeters of chloroform were added to each litre. In bottling the serum for use the supernatant serum was drawn off and the lower portion, containing sediment and excess of chloroform, discarded.

The material from the cases was examined both for diagnosis and in the hope that the cultures isolated could be studied for their complement fixing power, with the serum used, and that the protection value of the serum could be tested upon the type of pneumococci found. This study was not made, partly because of other work and partly because the strains were found to possess such low virulence for rabbits. When the intravenous inoculation of the growth from one blood agar slant was given only two strains killed, one in forty-eight hours and the other in ten days. Culture isolated from the heart's blood of these rabbits did not kill other rabbits, using a somewhat smaller dose. The sputum from Cases 18 to 23, inclusive, was examined. They all showed an overwhelming preponderance of typical pneumococci. One case of the series showed the presence of an abundance of streptococcus mucosus.

TABLE I

Case No.	Sex, Age in Years, Habits, etc.	Condition of Patient				Blood Culture, etc.	Serum Administration			Outcome of Disease		
		Day of Admiss'n	Type of Disease Invasion	Temp. Pulse, Resp.	Site of Lesion		Day of Disease	Dose Methods	Effect	Termination of Case	Serum Sickness and Day of Disease It Appeared	Remarks
1	M., 12	3	Chill	T. 104°-5° P. 132 R. 28	L. L.	Sterile	4	50 c. c., Intrav.	Chill T. 3° +	Crisis, 4th day. Recovery	Purpuric rash, arthritis. 8th day	Crisis a few hours after. serum
2	F., 1¼	15?	Chill	T. 104.8° P. 160 R. 50	R. U. and L. U.	.....	15 16 17	30, Subc. 30, " 30, "	.....	Crisis 18th and 19th day. Recovery	.....	1st and 2d injections without effect
3	M., 41 Alcoholic	8	Chill	T. 103° P. 124 R. 48	R. M. and L.	.....	8 9 10 11	100, Intrav. 100, " 100, " 100, "	T. 2° +	Resolution, death 19th day. Temp. rose 4°, oedema of lungs	.....	Pt. alcoholic, acute dilerium, oedema of lungs. Serum without effect
*4	F., 5	6	.....	T. 106° P. 140 R. 48	R. U.	.....	6 7 9 11 14	30, Subc. 50, " 45, " 50, " 50, "	T. 2° — T. 3° —	Death	Erythema, 19th day	Serum without any definite effect. Pt. very ill on admission
†5	M., 26	2	Chill	T. 106° P. 116 R. 28	L. L.	Sterile	3 4 5 5 6 7	100, Intrav. 100, " 100, " 100, " 150, " 200, "	.....	Recovery	Urticaria fever, 17th day	No definite effect of serum. Temp. fell by lysis after last injection
‡6	M., 17	4	Chill	T. 105° P. 128 R. 40	R. L.	Sterile	4 7 8 10	100, Intrav. 150, " 200, " 200, "	T. 2° — T. 3° +	Recovery	.....	Temp. fell gradually after 1st injection. No effect from later injections

7	M., 30	5	No definite Chill	T. 103.6° P. 108 R. 32	R. U. and M.	.....	6	200, Intrav.	T. 3.5° —	Recovery	Erythema 16th day	Sharp drop in T., P. & R. immediately after serum
8	M., 30	2	Chill	T. 105° P. 112 R. 24	R. U.	Sterile	3	80, Intrav.	Oedema of lungs in ½ hour	Crisis, 9th day. Recovery	26th day, T. 104°. 27th arthritis	Pt. had symptoms of anaphylaxis. Serum stopped no effect
9	F., 29	2	Cough and Pain	T. 103° P. 140 R. 40	R. L.	Sterile	4 6	150, Intrav. 175, "	.....	Recovery	.....	Resolution 4th week, no effect fr. serum
10	M., 46	7	Chill	T. 104.6° P. 108 R. 32	L. L.	Sterile	9	200, Intrav.	.....	Recovery	.....	Temp. fell by quick lysis after serum
11	M., 39 Alcoholic	6	.....	T. 104° P. 100 R. 36	L. U. and L.	Pneumococcus	6	200, Intrav.	.....	Death	.....	No effect of serum on T., P. & R. Died 3 days later
12	M., 16 In extremis	2	.....	T. 107°	R. L.	.....	3	150, Intrav.	.....	Death	.....	Died in 18 hrs.
13	F., 2	5	.....	T. 105° P. 136 R. 40	L. L.	.....	8	100, Intrav.	.....	Crisis 12th day. Recovery	Erythema 14th day	No effect from serum. Crisis 4th day after serum
¶14	M., 38 Alcoholic 3d attack	6	.....	T. 103.6° P. 124 R. 24	R. U.	.....	6	150, Intrav.	T. 2° —	Death	.....	Temp. rose steadily, died 11th day
15	M., 24	8?	.....	T. 105.4° P. 102 R. 32	R. L.	Sterile Plasmodium Malariae	9 11	250, Intrav. 50, Subc.	T. 4½° —	Death	.....	Temp. fell after 1st injection, but rose again and rose after 2d injection
16	M., 24	3	Chill	T. 105° P. 144 R. 40	L. L.	Sterile	4 5	250, Intrav. 50, Subc.	T. 3° —	Death	.....	Temp. dropped after 1st injection and then rose. No effect of serum

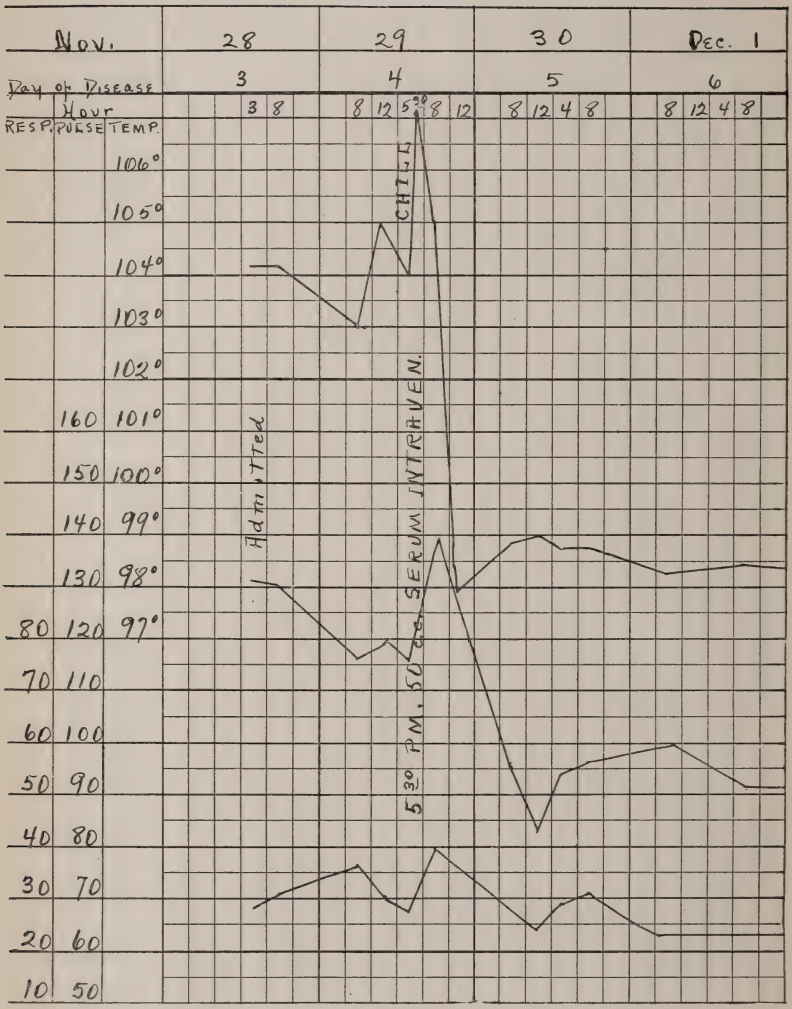
TABLE I —CONTINUED

Case No.	Sex, Age in Years, Habits, etc.	Condition of Patient				Blood Culture, etc.	Serum Administration			Outcome of Disease		
		Day of Admiss'n	Type of Disease Invasion	Temp. Pulse, Resp.	Site of Lesion		Day of Disease	Dose Methods	Effect	Termination of Case	Serum Sickness and Day of Disease It Appeared	Remarks
17	F., 4½	11	Chill and Cough	T. 101° P. 118 R. 32	L. L.	.....	12	50, Subc.	T. 2° —	Lysis Recovery	.....	Temp. fell 2° and then to normal after serum
18	M., 30 Alcoholic	6	.....	T. 105° P. 128 R. 44	L. U.	.....	6 9	250, Intrav. 50, Subc.	.....	Lysis Recovery	13th day rash and fever, 16th, arthritis	Slow lysis interrupted by serum sickness
§19	M., 32	4	Chill	T. 104.6° P. 120 R. 44	L. L.	.....	4 7	250, Intrav. 50, Subc.	.....	Lysis Recovery	Arthritis, fever 18th day	Serum without apparent effect
20	M., 13	3	Chill	T. 102° P. 120 R. 48	R. L.	Sterile	4	250, Intrav.	General Pains	Crisis 5th to 6th day. Recovery	Urticaria after two hours	Crisis 24 hours after serum
21	M., 54	6	Chill	T. 104.8° P. 88 R 24	R. U. and L.	Sterile	8	300, Intrav.	.....	Lysis Recovery	.....	Lysis rapid, 24 hours after serum
22	M., 20	5	Chill	T. 105.6° P. 120 R. 44	L. L.	.....	6	250, Intrav.	.....	Crisis 6th to 7th day. Recovery	.....	Crisis 9 hours after serum.
23	M., 33 Alcoholic	4	.....	T. 102.4° P. 100 R. 48	R. L.	.....	5	200, Intrav.	Nausea, vomiting, pulse rapid and sweating	Crisis 6th day. Recovery	.....	Crisis 24 hours after serum

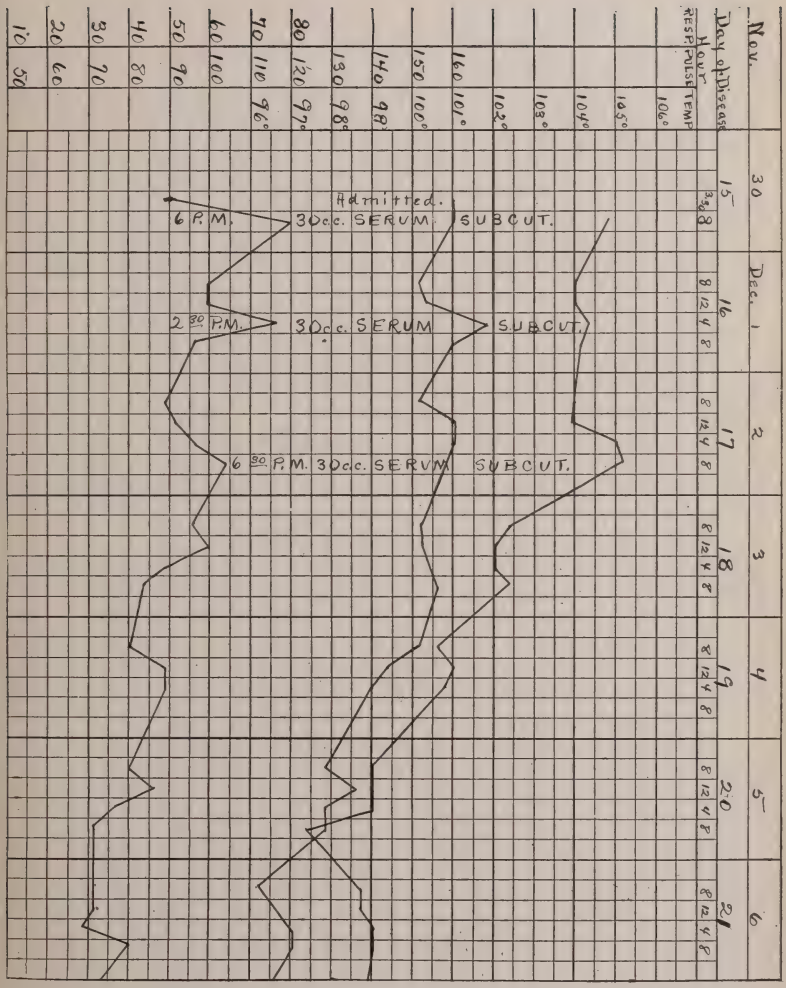
*Abscess of scalp, otitis media 21st day pyopneumotherax, 600 c. c. pus. Oedema of lungs 24th day.
†Pleurisy with effusion 150 c. c. aspirated.
‡Empyaema (pneumococcus) operation.
¶Oedema of lungs.
§Pleurisy with effusion, 10th day, 700 c. c. turbid serum. 12th, 350 c. c.; 25th, pus.

TABLE 2

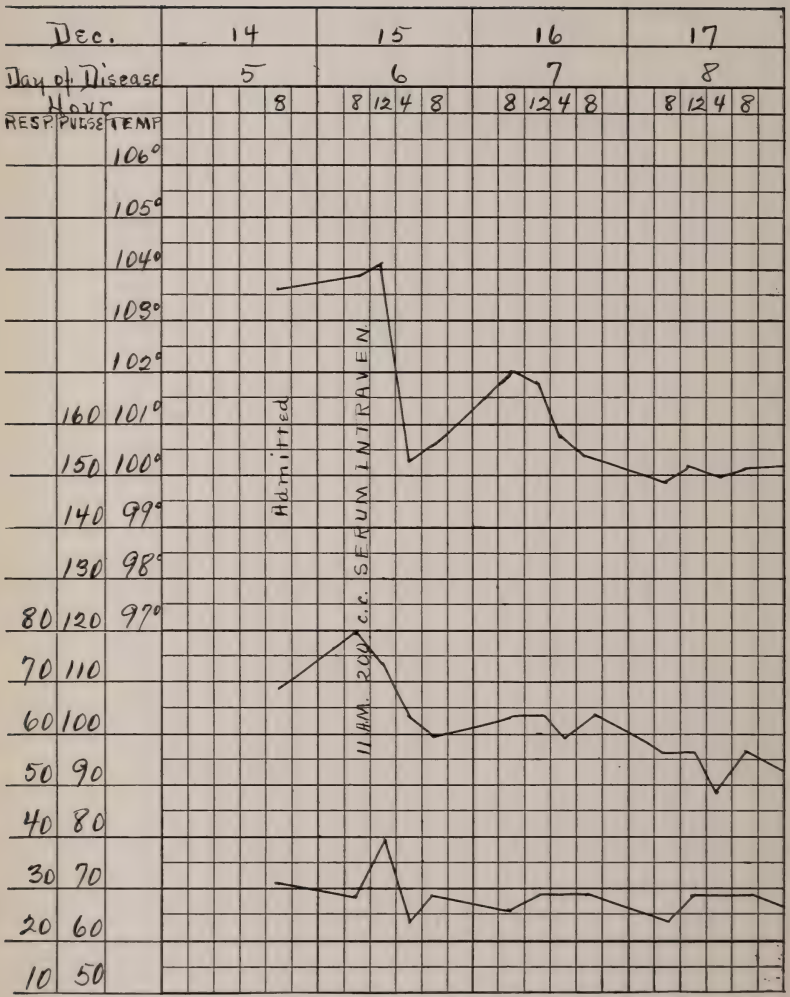
Case No.	Dose of Serum	Serum, c. c.	Leucocyte Counts					
			Before Serum		After Serum			
			Number of Cells	Per cent. of Polynuclears	Hours After Serum	Number of Cells	Per cent. of Polynuclears	Note
1	1st	50, Intrav.	25,000	86.5	2	19,000	85	
					4	30,000	87	
					6	29,000	81	Crisis
					18	20,000	69.5	
					27	14,000	44.7	
2	1st	30, Subc.	15,000	81	3	14,500	65	
					5	12,700	70	
					14	10,500	76	
	3d	30, Subc.	13,000	70	2	12,900	70	
					13	11,000	70	
					36	15,900	71	
3	1st	100, Intrav.	50,000	94.5	2	49,500	95	
					4	50,000	91	
					16	55,000	97	
5	1st	100, Intrav.	24,400	85	2	26,900	92.5	
					4	25,000	86	
					6	25,400	89	
					8	25,900	88	
					10	24,500	88	
					21	20,000	85	
6	1st	100, Intrav.	27,800	88.5	2	29,000	91.5	
					4	26,900	94	
					6	28,800	93	
10	1st	200, Intrav.	20,800	93.5	2	29,000	94	
					4	27,000	93	
					6	23,000	90	
					18	21,100	..	
16	1st	250, Intrav.	14,000	86	2	16,400	92	
					4	15,400	89	
					6	12,200	84	


Nov.
28
29
30
Dec. 1
Day of Disease
3
4
5
6
Hour
3 8
8 12 5:30 8 12
8 12 4 8
8 12 4 8
RESP. PULSE TEMP.
106°
105°
104°
103°
102°
160 101°
150 100°
140 99°
130 98°
80 120 97°
70 110
60 100
50 90
40 80
30 70
20 60
10 50
Admitted
CHILL
5:30 P.M. 50 cc. SERUM INTRAVEN.

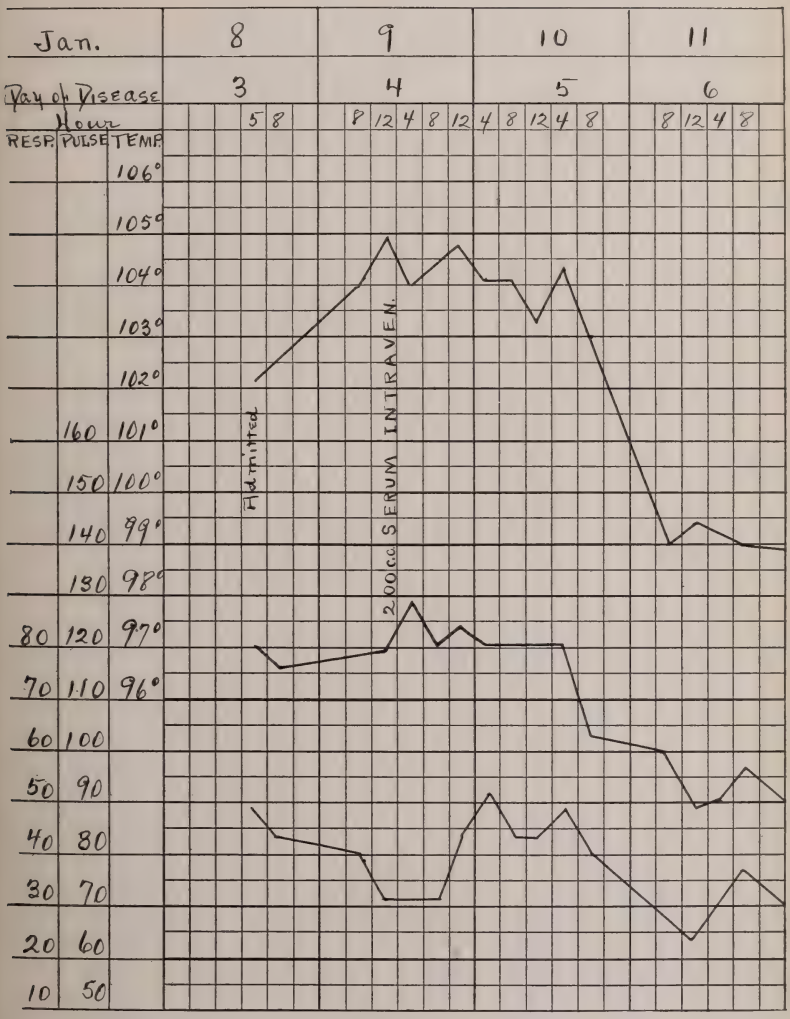
Case No. 1.


Nov.
30
Dec. 1
2
3
4
5
6
Day of Disease
15
16
17
18
19
20
21
Hour
3 8 8 12 4 8
RESP. PULSE TEMP
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
Admitted.
6 P.M. 30cc. SERUM SUBCUT.
2 30 P.M. 30cc. SERUM SUBCUT.
6 30 P.M. 30cc. SERUM SUBCUT.
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160

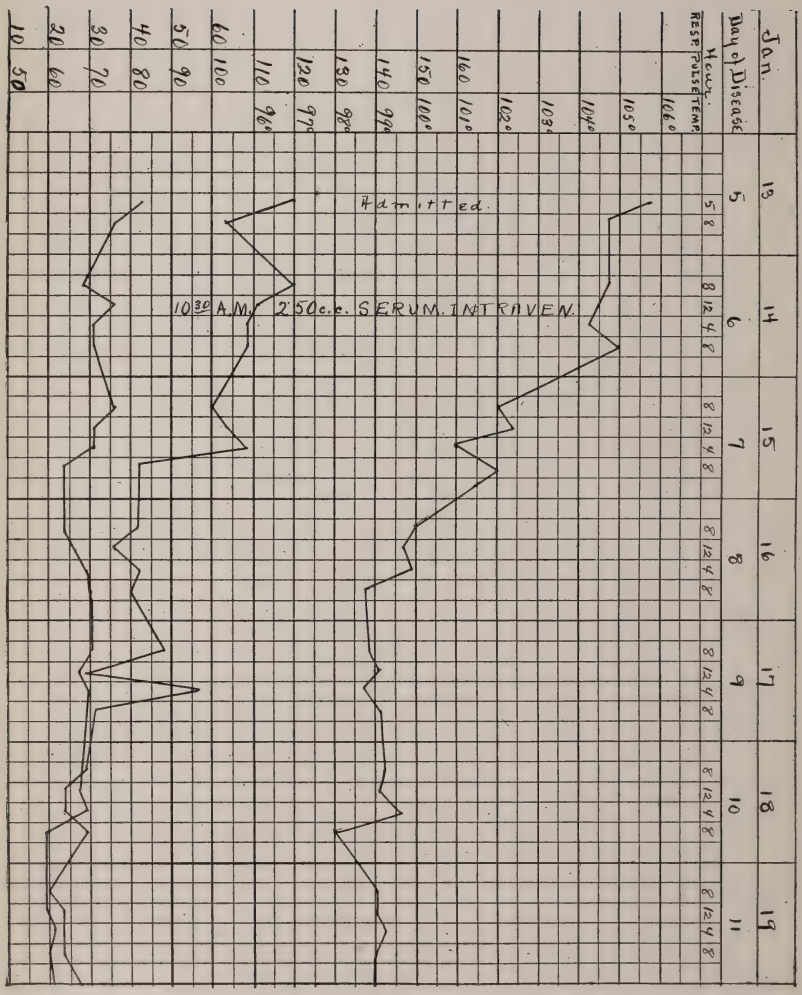
Case No. 2.


Dec.
14
15
16
17
Day of Disease
5
6
7
8
Hour
8
8 12 4 8
8 12 4 8
8 12 4 8
RESP. PULSE TEMP
106°
105°
104°
103°
102°
160 101°
150 100°
140 99°
130 98°
80 120 97°
70 110
60 100
50 90
40 80
30 70
20 60
10 50
Admitted
11 AM. 20 c.c. SERUM INTRAVEN.

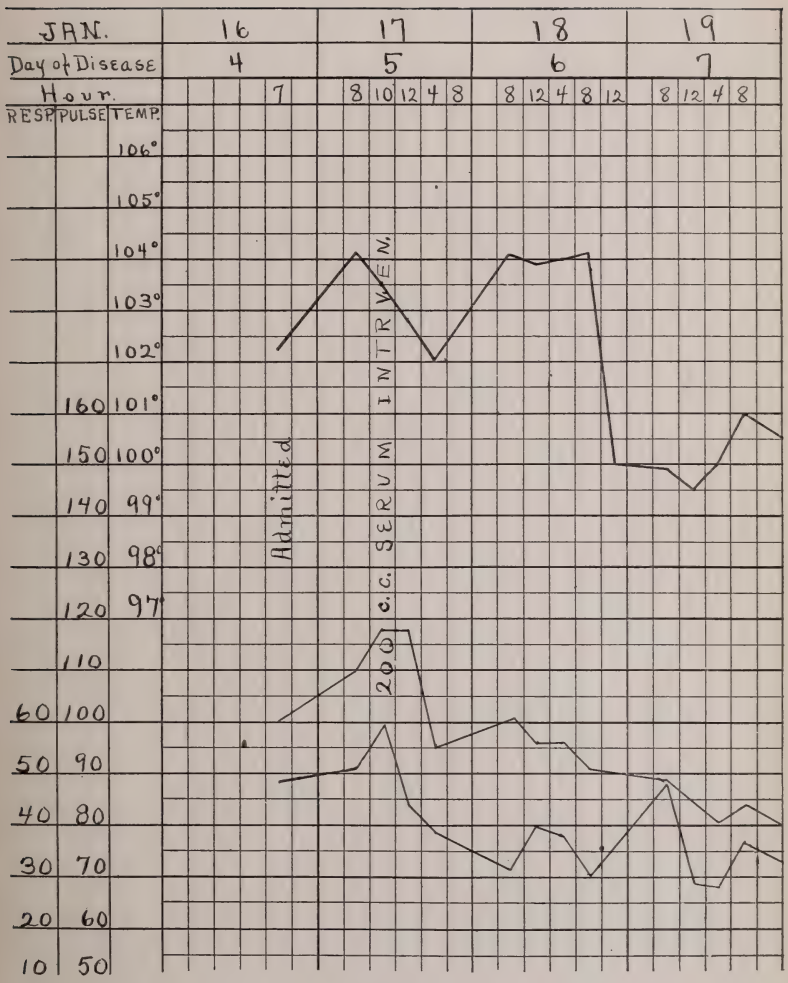
Case No. 7.


Jan.
8
9
10
11
Day of Disease
3
4
5
6
Hour
5 8
8 12 4 8 12 4 8 12 4 8
8 12 4 8
RESP. PULSE TEMP.
106°
105°
104°
103°
102°
160 101°
150 100°
140 99°
130 98°
80 120 97°
70 110 96°
60 100
50 90
40 80
30 70
20 60
10 50
Admitted
200 cc. SERUM INTRAVEN.

Case No. 20.


Jan.
Day of Disease
Hour
Resp. Pulse Temp.
Hatfitted.
10.30 A.M. 250 c.c. SERUM. INTRAVEN.

Case No. 22.


JAN.
16
17
18
19
Day of Disease
4
5
6
7
Hour.
7
8 10 12 4 8
8 12 4 8 12
8 12 4 8
RESP. PULSE TEMP.
106°
105°
104°
103°
102°
160 101°
150 100°
140 99°
130 98°
120 97°
110
60 100
50 90
40 80
30 70
20 60
10 50
Admitted
200 c.c. SERUM INTRAVEN.

Case No. 23.

A METHOD OF OBTAINING CULTURES FROM THE DUODENUM OF INFANTS

Alfred F. Hess

Ever since Escherich[1] published his classic monograph on the flora of the intestine during infancy, this field has been a favorite one for bacteriologists. They have, however, found themselves hampered not only by the difficulties attendant on growing intestinal bacteria, but by the difficulty of isolating pathogenic organisms from the multitude of saprophytic bacteria which flourish in the lower intestinal tract. Bearing this in mind, it seemed as if the duodenal catheter, devised primarily for the purpose of the diagnosis and treatment of pyloric stenosis, might be of value in this connection and afford a new method for the approach of this subject. This instrument is merely a soft rubber Nelaton catheter No. 14 or No. 15 (F) and marked in an appropriate manner. It can be inserted through the pyloric sphincter and into the duodenum in infants, from the time of birth up to the age of two years, with a facility comparable to the passage of the ordinary stomach-tube in the adult. It seems hardly necessary to outline the technic of this procedure, in view of the fact that a previous article[2] describes it in detail, including radiographs which clearly demonstrate the presence of the catheter within the lumen of the upper intestine.

For the purpose of bacterial investigation this duodenal catheter was sterilized and then capped at the end with a gelatin capsule, such as the apothecary frequently makes use of in dispensing drugs. This capsule had been sterilized by dry heat at 160 degrees C. and then, by means of sterile forceps, was slipped over the eye at the end of the catheter and fastened by slightly moistening with sterile water. The catheter thus prepared was passed in the usual way into the mouth and the stomach, and then introduced gently through the pylorus into the duodenum. In general the pylorus can be traversed by this means within three to five minutes. It was found that the capsule, which had become hardened, due to the heat of sterilization, did not melt before it reached the duodenum; this was readily ascertained by means of aspiration, for as soon as the end of the catheter is open, a drop or two of gelatin can be aspirated, whereas as long as it is closed, a marked sense of resistance to aspiration is encountered. By means of this technic it is possible to obtain the contents of the duodenum and of the jejunum, by the aid of antiperistalsis, with but very slight contamination from the mouth or the stomach. It is probable that a slight degree of contamination generally results, but, judged from what follows, this could not have occurred to a considerable degree. As an additional safeguard, the gelatin covering the eye of the catheter, which might constitute a source of contamination, was not sucked up with the first aspiration, but, on the contrary, was blown off the end of the tube.

[1]Die Darmbakterien des Säuglings, 1886.
[2]Am. Jour. Dis. Children, 1912, 3, p. 133.

The first tests were made upon new-born babies, a few hours old, before they had taken any food whatsoever. This group consisted of four cases. Very few bacteria were found in the stomach and in the duodenum. Only one to three bacteria to a platinum loop of the fluid. These were staphylococci, molds, gram-positive and, less often, gram-negative bacilli. No colon bacilli were found.

Next, 11 babies were tested during the first week of life. In each case a test of the stomach contents was first made, then one of the duodenal contents, the tests being carried out when the stomach was comparatively empty. Here, again, very few bacteria were found in the duodenum, and the same varieties were cultivated as in the new-born babies. As was to be anticipated, there was a general concordance between the bacteria of the stomach and those found in the upper intestine. In only one case of this series was the colon bacilli, or, indeed, any gas-producing organism, obtained; in these tests 0.3-0.5 c. c. of intestinal contents was cultivated. For comparison, a test of the mecomium was made for the presence of the colon bacillus. The earliest that this organism was met with was in the case of an infant 12 hours old. Generally it was not found in the mecomium. In this connection we may call attention to the work of Escherich, of Moro, and of Herter, who found the colon bacillus quite frequently in the mecomium, and therefore suggested that this bacillus first gains access to the gastro-intestinal tract by way of the rectum. The most common bacterium in the upper intestine during this period of life was the staphylococcus, in the stomach as well as in the duodenum. The bacteria seemed to be almost entirely of the facultative variety, for anaerobic cultures failed to show other organisms than those grown under aerobic conditions. It was found that the presence or absence of free hydrochloric acid in the stomach, or of a small or large quantity of bile in the intestine, did not play a constant rôle as regards the number of bacteria obtained. In tests made upon somewhat older infants, two to six weeks of age, the colon bacillus was almost regularly cultivated from the duodenum, and with a less degree of constancy from the stomach. However, these various tests are not considered conclusive and are given, not so much with the idea of furnishing a definite qualitative and quantatative test of the normal flora at this period of life, for we realize that they are insufficient in number to serve this purpose, but rather to show how this method may be made use of in this connection. The number of bacteria cultivated from the stomach was found almost always to be in excess of that met with in the duodenum, which, in itself, furnishes a conclusive argument that the duodenal cultures were not merely contaminations from the mouth and the stomach. The number of bacteria in the duodenal contents was at no time great. During the first few days of life hardly more were found than in the tests made before the infant had ever nursed. At this period and a little later, 25 to 100 bacteria were usually grown from a loop of intestinal contents, and even in infants several weeks of age not more than 100 to 200 organisms were cultivated per platinum loop. In the case of bottle-fed babies the number of bacteria was regularly higher. This

comparative sterility of the duodenum was not surprising in view of the work of Kohlbrugge and others on the auto-sterilization of the intestines. Some years ago bacteriological tests of the contents of the intestine of rabbits, which we undertook in connection with a study of antiperistalsis, also demonstrated this fact decisively.

In addition to these general considerations, we should like to mention two specific instances where these tests seemed to be of clinical interest. One was the case of an infant a week old, who had gastro-intestinal symptoms, coated tongue, some vomiting, and green stools; in this instance repeated tests showed a large number of staphylococci in the stomach, and a still larger number in the duodenum.

The opportunity presented itself of passing the catheter upon a baby 22 months old, suffering from typhoid fever in the beginning of the third week and giving a positive Widal reaction. A small amount of bile-containing fluid was aspirated from the duodenum, and from this fluid typhoid bacilli were cultivated in very large numbers. The marked success in this instance strengthened the idea that this simple procedure might be of value in this connection in the diagnosis of typhoid carriers. Although this catheter cannot be used upon adults, as it does not readily pass through the pylorus where the stomach is large and the sphincter is resistant, a duodenal tube, as devised by Gross or by Einhorn, which in the course of a few hours is carried into the duodenum by means of peristalsis, might well be used for this purpose. This tube is very thin and causes very little inconvenience, as I have had opportunity to observe. The advantage of a duodenal test for typhoid bacilli, over a test of the stool, is considerable, as the duodenum is the estuary for typhoid bacilli. In the intestine by far the greatest number of typhoid bacilli are found in the duodenum and the jejunum, as could be presupposed when we consider that they are in the blood at the very onset of the disease, that they are partly excreted from the blood into the bile, and having multiplied in this fluid are poured into the upper intestinal tract. In this connection we should also remember that one of the very last organs of the body that they forsake is the gall bladder, which is the favorite nidus of this bacillus within the body of the typhoid carrier (Chiari). A clear and conclusive conception of the incidence of the typhoid bacillus in the gall bladder and the various levels of the intestinal tract may be obtained from the table of Gaehtgens[1] which is appended (Table 1). From this table, which gives the results of cultures taken post mortem in cases of typhoid and typhoid carriers, it is clear that the typhoid bacilli decrease in numbers in the intestinal tract as we proceed downward from the duodenal opening of the bile duct to the rectum, and that they are to be found most constantly in the gall bladder.

This idea of cultivating typhoid bacilli from the duodenal contents, rather than from the stool, is not entirely original. Some years ago Weber made an attempt of this kind on account of the difficulty which had been experienced by others in cultivating typhoid bacilli from the stools of carriers. Kayser,[2] of the Kaiserliche Gesundheitsamt, found

[1]Berl. klin. Wchnschr. 1912, 49, p. 296.
[2]Arb. a. d. k. Gsndhtamte., —— 1906, 24, p. 173.

in a series of cases, that three repeated examinations at long intervals failed to show typhoid bacilli, where later tests proved the individuals to be carriers, and therefore recommended as a safeguard that tests of carriers should be carried out every two or three months for a period of a year. In view of the inadequacy of stool examinations, Weber[3] made use of Volhard's oil breakfast, which consists in giving about 200 c. c of olive oil by mouth to incite a regurgitation of the duodenal contents into the stomach. He then attempted to cultivate typhoid bacilli from the stomach contents. In three patients, two of whom were typhoid carriers and one a paratyphoid carrier, a very large number of typhoid colonies were thus obtained, whereas they were cultivated but sparsely from the stool. Wherever bile was found in the stomach, typhoid bacteria were likewise found in large numbers. The only difficulty was that the duodenal contents could not always be obtained by this means.

We wish in this paper merely to present for consideration a method for the diagnosis and the study of bacterial conditions of the intestine. It is possible that this technic may also be of value in connection with diseases of known or unknown origin, associated with animal parasites, such as, for instance, amebic infection of the intestine or of the liver. It may also have an application in the study of the influence of the various food-stuffs upon the bacterial flora of the intestines. However, in addition to its practical value in the diagnosis of the typhoid carrier, at present its main field of usefulness would seem to be in the study of dysentery and enteritis, diseases which play such an important rôle in the morbidity and mortality of early life, and which it is difficult to investigate satisfactorily by the aid of cultures from the stool on account of the vast number of contaminating bacteria. This difficulty is not encountered in cultures taken from the upper part of the intestinal tract.

TABLE I

Post Mortem Cultures of Typhoid Cases and Carriers, Showing Incidence of B. Typhosus in Gall Bladder and Various Levels of the Intestine

	Gall Bladder		Duodenum		Jejunum		Ileum		Colon		Rectum	
	+	—	+	—	+	—	+	—	+	—	+	—
1st Week.....	..	..	..	1	..	1	..	1	..	..	..	1
2d "	2	1	2	..	2	4	1	2	1	1	..	2
3d "	6	..	2	2	2	2	2	3	1	7	..	..
4th "	2	..	2	1	3	2	1	3	..	2	..	4
5th "	2	..	..	..	1	1	..	1	1	1	..	..
6th "	..	..	..	..	..	..	..	..	..	..	..	..
7th "	2	..	..	..	1	1	..	2	..	2	..	..
8th "	1	..	..	..	1	..	..	..	..	1	..	..
Total.....	15	1	6	4	10	9	4	12	3	14	0	7
Per cent.....	94		60		53		25		18		0	

\+ = Typhoid bac.
— = No typhoid bac.

[3]München med. Wchnschr., 1908, 55, p. 2443.

THE RELATION OF THE VIRULENCE OF THE TUBERCLE BACILLUS TO ITS PERSISTENCE IN THE CIRCULATION

Alfred F. Hess

Bacteria frequently gain access to the circulation. However, there is still a marked difference of opinion as to how frequently this occurs and how serious this invasion is, some believing that it is a phenomenon always accompanied by marked systemic disturbance, others that if the number of bacteria is small, the body rids itself of the invaders without the aid of any general reaction. It is known that micro-organisms are eliminated from the circulation chiefly by way of the kidneys and of the liver, but it is not known what the determining factors are. Are the bacteria filtered from the blood stream by means of the tissues, in the same way as inert foreign particles; for example, as would be the case if an equal amount of egg white had found its way into the blood, or does this mechanical process play a rôle secondary to other finer defensive processes?

The following investigation was undertaken primarily to obtain some answer to a simple question, to discover whether, quite independently of their power of multiplication, a direct relationship exists between the virulence of bacteria and their ability to maintain an existence in the circulation. It seemed as if the tubercle bacillus presented an unusual opportunity for a study of this nature. For, as is well known, the bovine type of tubercle bacillus is highly virulent for rabbits when a small fraction of a milligram is injected intravenously, whereas the human type of this bacillus is but slightly virulent, possessing the power of giving rise to merely local lesions in the lungs and in the kidneys, which do not desseminate the disease or result in the death of the animal. The two types of bacilli are almost alike morphologically, and multiply so slowly as to make this factor negligible in an experiment of short duration. Their chief and pre-eminent difference is that of virulence. A series of tests was therefore instituted, which, briefly stated, consisted in inoculating a measured quantity of human and of bovine tubercle bacilli into the circulation of rabbits, and of determining whether they persisted in the blood-stream for approximately the same length of time.

The experiments were planned as follows: Four rabbits were injected for each test, two with a human type of bacillus, the other two with the bovine type; in each case .01 mg. and 1 mg. of a culture was suspended in a salt solution and injected into the ear vein. These four animals, which were of about equal weight, were bled at regular intervals, generally one-half hour, one hour, two hours and three hours after inoculation, 5 c. c. of blood being caught in a solution of sodium citrate. Thus there were eight specimens taken from the two rabbits inoculated with the human tubercle bacillus, and eight from the two inoculated with the bovine bacillus. These sixteen specimens of blood were immediately injected subcutaneously into as many guinea-pigs, and after six weeks these animals were examined for tuberculosis.

In addition to a series of experiments of this description, others were undertaken in which the rabbits were bled soon after inoculation, or after a period longer than three hours following the injection of the bacteria. Furthermore, it was possible to test the blood of a large number of rabbits which, in the course of various laboratory work, had been inoculated with bovine or with human tubercle bacilli, and to study, in this connection, the relation of bacteria to the tuberculous condition of the animal—a subject which in the field of human pathology has been the centre of much discussion during the past few years, and which we touched on only incidentally.

It will be seen from the accompanying table (Table I) that six experiments, of the type which has been outlined, were successfully carried out. Others were undertaken, but owing to difficulty in bleeding the rabbits, to occasional clotting of the blood, to the premature death of some of the guinea-pigs, or to other unavoidable causes, they had to be altogether rejected.

The table shows our results more clearly than mere description. If we take the first experiment, for example, we find that of the eight pigs inoculated with the blood of rabbits injected with bovine tubercle bacilli, four showed tuberculosis when killed after an interval of six weeks, one died prematurely, so that three of the eight tests were negative. On the other hand, only one specimen of the eight taken from the two rabbits inoculated with the human culture incited tuberculosis in the guinea-pigs used for the tests. In this way the experiments may be interpreted. Table 2 brings out these results more clearly, and is a summary of all six experiments. Here we see that there were forty-four rabbits inoculated with a culture of human tubercle bacilli, and an equal number of successful inoculations with bovine culture. This includes all specimens of blood drawn at intervals varying from one-half to three or four hours after the rabbits were inoculated. Of the bovine tests eighteen were found to contain tubercle bacilli, that is, 39 per cent., whereas of the tests with human culture only four gave rise to tuberculosis in the guinea-pigs; that is, only a little over 9 per cent. It will be seen that there is some irregularity in the results; that at times bacilli were found in the circulation after two or three hours, although they were not demonstrable after shorter intervals. The difference between the two groups as shown in this table is, however, so marked that the conclusion is definite that the two types of bacilli, the markedly virulent and the feebly virulent, differ decidedly in their ability to persist in the circulating blood. That this difference is intimately associated with virulence was further brought out by an experiment in which a bovine culture was used, which was almost avirulent for rabbits as the result of prolonged artificial cultivation. (Table 1, Exper. II.) In this experiment, the bovine bacilli were not found in the circulating blood in any instance. Furthermore, it must not be thought that the negative results of the various tests with the human culture were attributable to its having lost its virulence for guinea-pigs, for a series of tests using from .001 mg. to .000000001 (1/1,000,000,000) mg.

of culture proved that .00001 (1/100,000) mg. incited a marked tuberculosis when injected subcutaneously.

TABLE 1

RELATIVE PERSISTENCE OF THE HUMAN AND THE BOVINE TUBERCLE BACILLUS IN THE CIRCULATION

Interval in hours between inoculation and bleeding	Amount and Type of Bacilli Injected into Rabbits				Remarks
	.01 mg. (B.)	1 mg. (B.)	.01 mg. (H.)	1 mg. (H.)	
Experiment I.					
1	—	Tb.	—	—	
2	—	Tb.	Tb.	—	
3	0	Tb.	—	—	
4	Tb.	—	—	—	
Experiment II.					
½	—	—	Tb.	—	Bovine culture was
1	—	—	—	—	almost avirulent
2	—	—	—	0	for rabbits
3	—	—	—	—	
Experiment III.					
½	0	—	—	—	Suggests possible
1	Tb.	0	—	—	confusion between
2	Tb.	—	—	—	the two "bovine"
3	Tb.	—	—	Tb.	rabbits
Experiment IV.					
½	Tb.	Tb.	—	0	Clot interfered with
1½	Tb.	Tb.	—	0	fourth test of first
2¼	Tb.	Tb.	—	0	column and third
3¼	—	Tb.	—	—	test of third column
Experiment V.					
½	Tb.	Tb.	—	—	
1	Tb.	Tb.	—	—	
2	Tb.	—	—	—	
3	—	Tb.	—	—	
Experiment VI.					
½	0	Tb.	—	—	
1	Tb.	Tb.	—	—	
2	—	Tb.	Tb.	—	
3	Tb.	Tb.	—	—	

Tb. = Tuberculosis of guinea-pig; — = no tuberculosis; 0 = premature death, etc.; B. = Bovine; H. = Human.

Table 3 is a record of bleedings of a rabbit made at various intervals for more than twenty-four hours after it was inoculated with 1 mg. of a virulent bovine culture. It will be seen that these bacilli were still

circulating in the blood twenty-seven hours after inoculation. In tests instituted one week to two months after inoculation with a bovine

TABLE 2

SUMMARY OF SIX EXPERIMENTS OF TABLE I

Experiment	Bovine Tb.		Human Tb.	
1	4	3	1	7
2	0	8	1	6
3	3	3	1	7
4	7	1	0	5
5	6	2	0	8
6	6	1	1	7
	+	—	+	—
	26	18	4	40
Total No. of cases	44		44	
Tb. per cent	59		9.1	

+ = rabbit's blood contained tubercle bacilli.
— = rabbit's blood did not contain tubercle bacilli.

TABLE 3

PERSISTENCE OF BOVINE TUBERCLE BACILLI IN THE BLOOD (*27* HOUR TEST*)

Time of Bleeding	Interval after Inoculation, Hours	Guinea-pig Test	Time of Bleeding	Interval after Inoculation, Hours	Guinea-pig Test
2 p. m.	2	Tb.+	9 a. m.	21	Tb.+
3 p. m.	3	Tb.+	11 a. m.	23	Tb.+
4 p. m.	4	Tb.+	1 a. m.	25	Tb.+
5 p. m.	5	Tb.+	3 a. m.	27	Tb.+

* 1 mg. of bovine culture in 1 c. c. of salt solution was inoculated into a rabbit. Five c. c. of blood tested.

culture (Table 4 A), tubercle bacilli were found in six out of seventeen instances, and all six had been injected from one to two months previously. So that it would seem that there exists a period in which the blod is sterile, an intermediate period between the time when the bacilli originally inoculated are eradicated from the blood-serum, and when in the course of a progressive tuberculosis, bacilli once more gain access to the general circulation. Among twenty-three similar tests in which a culture of human tubercle bacilli was inoculated, producing, however, but slight or no tuberculous lesions, only two showed bacilli in the blood (Table 4 B). In one of these positive cases a careful post mortem examination of the rabbit failed to reveal any tuberculous lesion; we regard the finding of bacilli in this instance as an interesting but chance occurrence.

TABLE 4

Later Tests of Inoculated Rabbits

A. Bovine

No. of Rabbit	Interval Since Inoculation, Days	Amount of Blood Tested, c. c.	Autopsy of Rabbit	Result of Blood Test
1148	7	7	Tb.	Tb.
1149	7	7	Tb. (m.)	Neg.
1149	14	7	Tb. (m.)	Neg.
1149	16	7	Tb. (m.)	Neg.
1153	14	7	Tb. (m).	Neg.
1153	22	6	Tb. (m.)	Neg.
1155	21	6	Tb.	Neg.
1155	29	6	Tb.	Neg.
1152	30	7	Tb.	Neg.
1100	30	4.5	Tb.	Tb.
1100	30	4.5	Tb.	Tb.
1127	30	..	Tb.	Tb.
1126	30	..	Tb.	Tb.
1126	42	5	Tb.	Neg.
1160	35	7	Tb.	Neg.
1159	60	10	Tb.	Tb.
1159	60	10	Tb.	Tb.
		B. Human		
1158	1	5	....	Neg.
1109	17	3	Neg.	Neg.
1109	20	6	Neg.	Neg.
1110	17	3	Tb.*	Tb.
1110	20	6	Tb.*	Neg.
1110	60	..	Tb.*	Neg.
1105	17	3	Tb.*	Neg.
1105	20	6	Tb.*	Neg.
1104	17	3	Tb.*	Neg.
1104	24	6	Tb.*	Neg.
1107	17	3	Tb.*	Neg.
1107	24	6	Tb.*	Neg.
1103	17	3	Neg.	Neg.
1103	24	6	Neg.	Neg.
1108	17	3	Neg.	Neg.
1108	24	6	Neg.	Neg.
1118	30	5	Neg.	Tb.
1118	90	3.5	Neg.	Neg.
1118	90	10	Neg.	Neg.
1121	30	5	Neg.	Neg.
1121	90	3.5	Neg.	Neg.
1121	90	3.5	Neg.	Neg.
1161	30	7	Neg.	Neg.

m = Miliary tuberculosis. The other rabbits had typical, massive bovine tuberculosis.

* Slight.

It is difficult to form a satisfactory hypothesis to explain the mechanism by which bacteria are able to circulate hundreds of times within the blood-stream and persistently escape the tissue filters. The feebly virulent human type, as well as the bovine bacillus, was regularly found in the blood from three to twenty minutes after inoculation (Table 5).

TABLE 5

Early Bleedings of Inoculated Rabbits

Nature of Inoculation	Interval After Inoculation, Minutes	Amount of Blood Tested, c. c.	Result
Bovine (1 mg.)	3	5	Tb.+
Bovine (1 mg.)	10	5	Tb.+
Bovine (1 mg.)	20	5	Tb.+
Human (1 mg.)	3	5	Tb.+
Human (1 mg.)	10	5	Tb.+
Human (1 mg.)	20	5	Tb.+

It is possible that the bacilli cling to the walls of the vessels and escape the current of the circulation for considerable periods. However, in view of the lack of experimental data in this particular it is best to forego hypotheses. This is likewise true in regard to efforts attempting to account for the difference in this respect between the virulent and the comparatively avirulent types of tubercle bacilli. It is not attributable, however, to a difference in the opsonic power of the blood; tests which we carried out, as well as those of others,[1] show that no clear distinction exists between the indices for the human and for the bovine types of tubercle bacilli.

In the course of the inoculation of rabbits' blood into guinea-pigs, interesting toxic (anaphylactic) phenomena were frequently observed—abdominal distension, coma, spasm of the hind legs, scratching of the face and other symptoms of this complex which are well known. These symptoms have been described by others,[2], following intraperitoneal inoculation of rabbit serum, as well as local necrosis,[3], which, however, we did not encounter in the course of our many subcutaneous injections. It seemed to us as if the toxic symptoms were more frequent and more marked in instances in which blood was injected from rabbits suffering from bovine tuberculosis. Accordingly we undertook an extended series of tests, summarized in Table 6, to gain a clearer understanding of this reaction. It became evident that our impression was correct, that pigs injected with normal blood or the blood of rabbits inoculated with a culture of the human tubercle bacillus reacted less regularly and with less intensity than did those inoculated with an equal amount of the blood of rabbits suffering from tuberculosis of the bovine type. Guinea-pigs inoculated with the blood from the bovine rabbits frequently

[1]Koehlisch: Ztschr. f. Hyg., 1911, lxviii, 193.
[2]Thomson, O.: Ztschr. f. Immunitatsf., 1909, i, 741.
[3]Pfeiffer: Ztschr. f. Hyg., 1897, xxvi, 384.

TABLE 6

ANAPHYLACTIC SYMPTOMS FOLLOWING SUBCUTANEOUS INJECTION OF RABBITS' BLOOD

Condition of Rabbit	Amount of Blood Injected, c. c.	Anaphylactic Symptoms	Death	Remarks *
Bovine Tb...	5	++	+	Died in ½ hour
Bovine Tb...	5	—	..	
Bovine Tb...	5	++	+	
Bovine Tb...	5	++	..	
Bovine Tb...	5	++	..	
Bovine Tb...	5	++	..	
Bovine Tb...	5	++	+	Died in 1½ hours
Bovine Tb...	5	++	+	Died in 1½ hours
Bovine Tb...	5	+	..	
Bovine Tb...	5	+	..	
Bovine Tb...	5	—	..	
Bovine Tb...	3.5	++	..	
Bovine Tb...	5.5	+	..	Blood in refrigerator over night
Bovine Tb...	7	++	+	Death in 28 hours
Bovine Tb...	7	++	+	Blood in refrigerator nine days
Bovine Tb...	7	++	+	Blood in refrigerator nine days
Bovine Tb...	10	++	..	
Bovine Tb...	14	++	..	
Human Tb..	5	—	..	Rabbits almost free from tuberculous lesions
Human Tb..	5	—	..	Rabbits almost free from tuberculous lesions
Human Tb..	5	—	..	Rabbits almost free from tuberculous lesions
Human Tb..	5	—	..	Rabbits almost free from tuberculous lesions
Human Tb..	5	—	..	Rabbits almost free from tuberculous lesions
Human Tb..	7	++	..	Rabbits almost free from tuberculous lesions
Human Tb..	7	+	..	Rabbits almost free from tuberculous lesions
Normal.....	5	+	..	
Normal.....	5	—	..	
Normal.....	5	—	..	
Normal.....	5.5	—	..	
Normal.....	7	+	..	
Normal.....	7	+	..	
Normal.....	7	—	..	
Normal.....	7	—	..	
Normal.....	10	++	..	
Normal.....	10	—	..	
Normal.....	17.5	++	..	

* Guinea-pigs weighed about 250 gm.

died with symptoms simulating anaphylactic shock. Serum stored in the refrigerator for some days retained this toxic property. The toxicity cannot be explained by the fact that in the one case we were testing the

blood of a markedly diseased animal, and in the other that of an animal which was almost normal, for the blood of rabbits suffering from rabies did not possess similar toxic properties. In this connection it should be mentioned that Friedberger and Schuetze[4] were able to extract anaphylatoxin in the test tube from tubercle bacilli, by adding normal guinea-pig serum and complement. The amount of toxin obtained varied according to the proportion of antigen, serum, and complement used, and the period of extraction. Our results constitute a confirmation *in vivo* of these test-tube experiments; their lack of absolute regularity must be attributed to the variability of the diverse controlling factors.

Conclusions

For an experiment such as we set ourselves, namely, to determine the relation of virulence of bacteria to their persistence within the blood-stream, the tubercle bacillus would seem to be especially suitable. Apart from the fact that it multiplies so slowly, that this factor is negligible in a test of short duration, it occurs in two types, one feebly virulent to rabbits the other highly virulent. Morphologically these bacteria are almost identical; their pre-eminent difference is one of virulence. Accordingly define quantities of these closely related micro-organisms were inoculated into rabbits, and bleedings were carried out from one-half to four hours later to discover whether bacilli were still circulating in the blood. In a series of tests of this nature it was found that the feebly virulent human type of bacillus was present in only 9 per cent. of the tests, whereas the virulent bovine bacillus persisted in the blood-stream in 59 per cent. of the cases. These results seem to warrant the conclusion that virulence plays an important rôle in bacteremia, and that the bacteria may not be filtered from the blood by the tissues like inert foreign bodies. The fact that an avirulent bovine strain did not persist in the circulation strengthened us in this conclusion.

It is remarkable for how long a period after inoculation bacteria may still be found in the general circulation. In one instance in which but 1 mg. of bovine culture was inoculated, these bacilli were constantly found in the blood at various intervals during the subsequent twenty-seven hours. A period intervenes some days after inoculation during which even the virulent organisms are not found in the blood. However, this constitutes merely an intermediate or latent phase, and is followed by another phase cycle, in which, owing to the tuberculous condition of the animal, there is a reinvasion of the general circulation. For example, in tests performed a week or two subsequent to inoculation no bacilli were found, whereas in tests repeated a month later, when systemic tuberculosis had developed, bacilli were frequently demonstrated. These generalizations are subject to exception; in the case of one animal, although tubercle bacilli were obtained from the blood, autopsy some weeks later failed to reveal any tuberculous lesion.

In the course of a large series of injections, the interesting and suggestive phenomenon was noted, that the blood of the highly tuberculous rabbit is more toxic for the ginea-pig than that of the normal rabbit.

[4]Friedberger, E. and Schuetze, A.: Ztschr. f. Immunitatsf., 1911, ix, 431.

ACID AGGLUTINATION OF TUBERCLE BACILLI*

By Charles Krumwiede, Jr.

Michaëlis, in the "Deutsche medizinische Wochenschrift." No. 21, 1911, calls attention to the fact that colloids and bacteria have specific points of precipitation in acid solutions, depending on the degree of hydrogen dissociation. The following table gives an example of the preparation of a set of solutions:

Solution	1	2	3	4	5	6
Acetic Acid $\frac{N}{1}$	7.5 c.c.	10 c.c.	15 c.c.	25 c.c.	45 c.c.	85 c.c.
Sodium Hydroxid $\frac{N}{1}$	5 c.c.	5 c.c.	5 c.c.	5 c.c.	5 c.c.	5 c.c.
Water	87.5 c.c.	85 c.c.	80 c.c.	70 c.c.	50 c.c.	10 c.c.
Ionization	1.10-5	2.10-5	4.10-5	8.10-5	16.10-6	32.10-5

The test is performed by making a suspension of the bacteria in distilled water. Three cubic centimeters are added to six tubes and the above six solutions added in 1 c.c. amounts. With typhoid and paratyphoid the following is an average reaction:

Solution	1	2	3	4	5	6
Typhoid	−	±	+	+	±	−
Paratyphoid	−	−	−	−	+	+

Michaëlis stated in his first examination that tubercle bacilli were alike in having their maximum agglutination at a hydrogen dissociation of 0.6 . 10-3, and this was the same for each type. He did not mention the range of agglutination, however.

In a later article Beniash ("Zeitschrift für Immunitätsforschung," vol. 12, p. 304) gives the following table. The mixtures were made with lactic acid and sodium lactate. The medium on which the bacilli were grown is not stated.

Ionization of Solution	1.7.10-5	3.5.10-5	0.7.10-4	1.38.10-4	2.76.10-4	5.5.10-4	1.1.10	2.2.10-3	4.4.10-3
Human 1	−	−	+	+	++	++	++	+	±
" 2	−	−	−	±	±	+	+	±	±
Bovine 1	−	−	±	±	++	++	++	±	±
" 2	−	−	−	−	±	±	±	−	−
" 3	−	−	−	±	±	+	++	±	±
Avian	−	−	−	±	±	+	±	−	−

* The introductory matter is given for convenience, although it is repeated somewhat more fully in another paper in these studies.

We had tried some preliminary tests with bovine and human strains, using the solutions given in the first table. These showed a difference in the range of agglutination. There were on hand the dried bacteria filtered off from heated broth cultures. These had been stored in the dry condition for several months up to a year or longer. Having only a limited number of fresh strains, the dried bacilli were ground up and suspensions prepared. As controls, fresh cultures, both heated and unheated, were used. Before making the suspension the bacilli were thoroughly washed with distilled water. The results were as follows:

Solution	1	2	3	4	5	6
Ionization	1.10-5	2.10-5	4.10-5	8.10-5	16.10-5	32.10-5
Bovine type:						
1 strain	−	−	−	−	+	+
3 strains	−	−	−	±	+	+
4 "	−	−	−	+	+	+
Control heated	−	−	−	+	+	+
" unheated	−	−	−	±	+	+
Human type:						
4 strains	−	±	+	+	+	+
7 "	−	+	+	+	+	+
2 "	±	±	+	+	+	+
12 "	±	+	+	+	+	+
Controls: 5 strains heated and unheated	Same range as preceding.					

These results held on repetition with some of the same suspensions. The readings were made after one to two hours at room temperature. Readings were controlled by allowing the tubes to stand on ice over night. After this they were gently inverted to rule out spontaneous sedimentation. A moderately thick but translucent suspension was used.

Meanwhile fresh cultures of the available strains were planted and tested. These results have been different and show no uniformity, even with the same strain on successive tests or with different cultures of the same strain. Where no mention of media is made, the cultures were on broth. When the results were found to be irregular, various factors were introduced to approximate the conditions in the first series, as drying, heating, or both. The dried tubercle bacilli had been washed by pouring distilled water on the masses of bacilli and changing once or twice daily for several days. It was thought that washing of the fresh cultures on filter-paper was not as thorough as this, and that the first test was really made with dialyzed bacteria. To approximate this some cultures were dialyzed.

The following table shows these irregularities sufficiently:

Solution	1	2	3	4	5	6	Notes
Ionization	1.10-5	2.10-5	4.10-5	8.10-5	16.10-5	32.10-5	
Human type: No. 305	−	−	+	+	+	+	Same cultures throughout.
" heated	−	+	+	+	+	+	
" dried	−	−	−	−	+	+	
" " and heated	−	−	−	−	−	±	
No. 470	±	+	+	+	+	+	Same cultures throughout.
" dried	−	−	−	−	−	−	
"	±	±	+	+	+	+	Same cultures throughout.
" heated	−	−	−	−	±	+	
" dried	−	−	−	+	+	+	
" " and heated	−	+	+	+	+	+	
Bovine type: No. 311	±	+	+	+	+	+	Same cultures throughout.
" dialyzed	−	±	+	+	+	+	
" " and dried	− −	− −	− −	+ −	+ +	+ +	
No. 536 dried	±	+	+	+	+	+	Same cultures throughout.
" " and heated	±	+	+	+	+	+	
Ravenel H.: Heated and unheated	+	+	+	+	+	+	Flocculation in control. Same result with filtered suspension.
Ravenel H.: Dried	−	−	−	+	+	+	Different culture than preceding.
No. 490	−	−	−	±	+	+	Same culture.
" heated	−	−	−	±	+	+	
"	−	−	±	+	+	+	Same culture.
" dialyzed	−	±	+	+	+	+	
" " and dried partly	−	−	−	+	+	+	
" dialyzed and dried	±	±	+	+	+	+	

The following are some tests of cultures from other media than broth:

Solution	1	2	3	4	5	6	Notes
Ionization	1.10-5	2.10-5	4.10-5	8.10-5	16.10-5	32.10-5	
Avian type: A. Glycerin-agar.....	−	−	−	−	−	±	Only after 15 hours.
" " "	−	−	−	+	+	+	
B. " "	−	−	−	−	±	±	
" Glycerin egg.....	−	−	−	−	±	+	
" " "	−	−	−	±	±	±	
" " "	−	−	−	−	−	±	Only after 15 hours.
C. Glycerin egg.....	−	−	−	−	−	±	Flocculation in 4 or 5 after 15 hours.
Human type: No. 667 (Glycerin egg)	−	−	−	+	+	+	
No. 814 (Glycerin egg)	−	−	−	+	+	+	
Dowd. (Glycerin egg)	−	±	+	+	+	+	
H. 1 (Glycerin egg)	−	−	−	−	±	±	Very slow.
(Glycerin egg)	−	−	±	+	+	+	
H. 3 (Glycerin egg)	±	±	±	±	±	±	
Bovine type: No. 8.....	−	−	−	−	−	+	
No. 490 (Glycerin egg)	−	−	+	+	+	+	
No. 123 (Glycerin egg)	−	−	±	+	+	+	
No. 122 (Glycerin egg)	−	−	+	+	+	+	Slight sedimentation in tubes 1 and 2.
B. 1 (Glycerin egg)	−	−	±	+	+	+	

Under one set of conditions with dried tubercle bacilli where the test was carried out as one series, a uniform difference was noted between the human and bovine types. With fresh cultures this difference disappeared. An attempt to approximate the original conditions gave varying results. Drying the bacilli had an influence on the range of flocculation, but on both human and bovine types. Heating gave irregular results. The only factor not tried was the time of storage of the dried bacilli. Whether these uniform results could be reproduced with old dried cultures cannot be answered. The fluctuation in range of the three types, with fresh cultures at least, is much the same and therefore of no value in differentiation.

OBSERVATIONS ON THE GROWTH OF BACTERIA ON MEDIA CONTAINING VARIOUS ANILIN DYES

CHARLES KRUMWIEDE, JR., AND JOSEPHINE S. PRATT

In recent communications Churchman (1) has described a selective action on bacteria of gentian violet. Stated in general terms, he found that bacteria were divided into two groups, of which one fails to develop in media containing gentian violet, while the other grows abundantly in the presence of this dye. The former group corresponds roughly with the Gram-positive group, the latter with the Gram-negative group of bacteria. His conclusions are based mainly on results obtained with dilutions of 1 to 100,000 of the dye.

A striking variation was noted in some enteriditis strains. These strains were identical by all ordinary cultural tests; one strain, however, persistently refused to grow on gentian violet agar. A subsequent examination showed that this strain could be differentiated from the others by agglutination.

Our interest in the use of media containing dye stuffs was further aroused by an article by Signorelli (2). He stated that on agar containing dahlia, cholera vibrios develop colored colonies by absorption of the dye with decolorization of the medium, whereas non-cholera vibrios give colorless colonies. We (3) were unable to verify these findings. In the course of the work we found two non-cholera vibrios which uniformly refused to grow on agar containing dilutions of dahlia which had no influence whatever on the growth of similar vibrios. Because of the above results, a few preliminary observations with various anilin dyes were made, which showed differences among related bacteria, and also differences in the reaction of the same bacteria to different dilutions of the same dye. The results of the comparison of the action of various dyes are given in this paper.

For the tests, the common pathogenic bacteria which grow freely on ordinary agar were selected. *Bacillus subtilis* was included as it is very sensitive to the presence of gentian violet. Other bacteria requiring serum or other similar additions were tried in a limited way, but this was discontinued as the albumins interfered with the activity of the dye.

As a routine a batch of agar was selected which was found especially suitable for the more feeble growing types. To the hot agar an appropriate volume of a watery solution of dye was added to give the final dilution desired. The same agar was used in each experiment.

The most convenient and economical method was found to be as follows. One unopened Petri dish was used to tilt up one side of a second dish. In the opposite side was poured just sufficient agar to give a satisfactory slant. This dish was covered and used to tilt up a third dish, and so on in a row. After the agar had set, slants were poured in the other side of the Petri dishes which were tilted in the reverse direction. In this way two mixtures of agar could be used in the same dish, very little agar being required for each slant.

In the general observations that follow two dishes were used containing the control agar, and agar with 1 to 500,000, 1 to 100,000, and 1 to 50,000 dilutions of the dye. The weakest dilution was used in the plate with the control.

For inoculation a fresh broth culture was usually employed. A small loop was used and a streak made on the agar slant, a fresh loopful being taken for each slant. Very heavy seeding as from agar slants tends to give irregular results, probably because of lack of close contact between the bacteria and dyed agar.

Table I gives the results of a series of tests.

Some allied bacteria showed variations as noted in the table; namely, the streptococcus-pneumococcus group, the dysentery group, the capsulatus group, and diphtheria and the morphologically allied types. More complete tests of these groups were made.

Naphthalamin blue R gave an apparent difference between diphtheria and diphtheroid bacilli. Thirty-four strains of these bacilli were, therefore, planted on agar containing this dye. There was irregular restraint at 1 to 100,000, more marked or complete in some instances at 1 to 50,000. There was, however, no evidence of a grouping according to the different types of bacilli.

On cresylecht violet the strain of pneumococcus failed to grow whereas other allied cocci grew. Forty strains of this group were tested. A variable restraint or inhibition of individual cultures was noted, but this had no relation to the subgroups.

In the dysentery group there is a general tendency for the paradysentery types to show less restraint than the Shiga type. The result on fuchsin seemed to show a definite difference between the types. The inhibition caused by fuchsin was also of interest because of its presence in Endo agar. This is the most commonly used medium for the isolation of dysentery and must, therefore, have been found satisfactory for this group; at least we know of no unfavorable criticism of the growth on this medium. The question arises as to how far the influence of the dye itself might be lost after its reduction by sodium sulphite to the leuko-base.

Table II. gives the results on fuchsin agar with the available strains of dysentery and paradysentery. The table gives the range of growth observed in several repetitions.

It is probable that some of the cultures under similar names, but obtained from different institutions,[2] are duplicates. As we were unable to determine this positively they are given separately in the table. They act as probable controls on the degree of variation of growth in the presence of the dye.

It is evident from Table II that the reaction to fuchsin is not a completely specific difference between the dysentery and paradysentery groups. There is a shading of one group into the other. There is, however, a marked tendency to a general group reaction.

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[2]We are indebted to the Museum of Natural History, to The Rockefeller Institute for Medical Research, and to Professor Hiss and Dr. Torrey for these cultures.

TABLE 1[1]

Type	Bacteria tested	Hoffman violet			Crystal violet			Dahlia			Fuchsin			Rosanalin violet			Cresylecht violett			Anilin violet. Gentian B			Naphthylamin blue R			Bleu de Lyon			Säureviolett. Kühne			Methylviolet 6 B		
		1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000	1:50,000	1:100,000	1:500,000
Gram-positive	Staphlycoccus aureus	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−*	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−
	Streptococcus (green)	−	−	+	−	×	+	−*	×	+	×	±	+	−	±	+	+	+	+	−	×	+	+	+	+	+	+	+	+	+	−	±	+
	Streptococcus (hermolytic)	−	−	±	−	−	±	−	−	±	−	−*	+	−	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−	+	+
	Streptococcus mucosus capsaulatus	−	−	+	−	−	±	..	..	..	−	×	+	−	−	+	+	+	+	−	×	+	+	+	+	+	+	+	+	−	−	+	+
	Pneumococcus	−	−	−	−	−	±	−*	−*	+	−	±	+	−	−	+	+	+	+	−	×	+	+	+	+	+	+	+	+	−	−*	+	+
	B diphtheriæ	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	+	+	−	−	−	±	±	+	+	+	+	−	−	−	−	−	−
	Diphtheroid bacilli (Cameron)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
	Diphtheroid bacilli (Cunningham)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
	B. hoffmanni	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	±	±	+	+	+	+	−	−	−	−	−	−
	B. subtilis	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−
	B. lactimorbi	−	−	−	−	−	−	−	−	−	−	−	+	−	−	±	×	±	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−*
	B. anthracis	−	−	−	−	−	−	−	−	−	−*	+	+	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−

| | | |

TABLE II

Growth of Dysentery Bacilli on Fuchsin Agar

Culture	Mannite fermentation	Time of Incubation and Dilution of Dye										
		18 to 24 hours				48 Hours				72 Hours		
		25,000	50,000	100,000	150,000	25,000	50,000	100,000	150,000	25,000	5,000	100,000
Shiga, Gay-Shiga, Shiga	—[3]	—	—	—	— or ×	—	—	s.c. or ×	±	—	s.c.	±
Kruse, Kruse	—	—	—	— or s.c.	— or ×	—	—	s.c. or ×	±	—	s.c. or ×	±
Kruse, Shiga	—	—	—	— or ×	×	—	—	× or ±	±	—	×	+
Shiga	—	—	—	— or ×	±	—	— or s.c.	× or ±	±	—	±	+
Torrey 21	—	—	—	× or ±	±	—	× or ±	+	+	— or l.c.	× or ±	+
Cruz 1	—	—	— or ×	± or +	+	— or ×	±	+	+	— or ×	±	+
Sydenham	+	—	— or ×	± or +	+	—	× or ±	+	+	—	±	+
Torrey 6, Torrey 5-6, Hiss Y	+	—	×	± or +	+	s.c. or ×	± or +	+	+	× or ±	±	+
Hiss Y, Flexner-Harris, Medburg, Ferra-Hiss, Torrey 16-3, Torrey 32, Y, Baltimore, Strong, Flexner, Mt. Dessert	+	×	± or +	± or +	+	× or ±	± or +	+	+	±	± or +	+
Strong, Gay-Flexner, Silk, Baltimore, Flexner, Gray, Torrey 3, Torrey 41, Flexner-Harris	+	±	± or +	+	+	± or +	+	+	+	± or +	+	+
Gray B, Hiss 48	+	+	+	+	+	+	+	+	+	+	+	+

[3]— = no growth; l.c. = colony; s.c. = several colonies; × = very slight growth; ± = evident restraint; + = practically like control.

Two strains, one of the Shiga type and the other of the paradysentery type, which reacted almost alike were plated and ten fishings of each made in broth. These were planted on fuchsin agar to determine the probable range of variation under identical conditions. A moderate variation was noticed in some instances slightly greater than those already noted in the table.

Influence of Decolorization on Fuchsin.—An agar was made containing decolorized fuchsin diluted 1 to 25,000. The strains showing no growth in this concentration of the unaltered dye after twenty-four hours' incubation, were planted. Three strains grew as well as on the control, four strains showed slight to moderate restraint, and one strain showed marked restraint. With a similar medium containing the decolorized dye in a dilution of 1 to 50,000, one strain showed some restraint, but at 1 to 100,000 no restraint was evident with any of the strains.

Evidently decolorization robs the dye of some of its action. A series of plates was made containing the decolorized dye in the 1 to 50,000 dilution. These were kept at room temperature and in the light to bring back the color gradually. Several of these plates, with controls, were planted each day. When planted the day after making, some restraint was evident. Those planted thereafter gave no growth except with a strain which grew in the presence of undecolorized fuchsin. The reaction of three members of the dysentery group to the violet dyes has been given. Because of the results on fuchsin the reaction of all the strains was tried, gentian violet and dahlia being used. With both dyes, individual strains showed more variation than with the fuchsin. There was no apparent difference between the dysentery and paradysentery group; the individual strains of both groups showing, however, markedly different degrees of restraint in the presence of the dye.

Reaction of the Streptococcus-Pneumococcus Group to Dyes.—In the results already given there is a marked quantitative difference in the reaction of the streptococcus-pneumococcus group as compared with the other Gram-positive bacteria. Because of this about a hundred strains of the group were tried,[4] these included typical pneumococci, streptococci, *Streptococcus mucosus,* types from chronic endocarditis, streptococci from various diseased conditions in man, a series isolated from the conjunctiva, a series isolated from domestic animals, and a few from the external world. Two dyes were used, gentian violet and Hoffman violet. The results were practically the same with both dyes.

In dilutions of 1 to 500,000 of the dye the members of the group grew as freely as on the control, with a few exceptions where slight restraint or delayed growth was evident. By increasing the concentration of the dye, the amount of restraint becomes greater, and some of the strains fail to grow, the number increasing with the concentration. When a dilution of 1 to 100,000 is reached a few strains still grow but the growth is restrained or delayed. One exception was a strain of *Strepto-*

[4]We are indebted to Miss Jean Broadhurst for most of the streptococcus cultures.

coccus lacticus, which even at 1 to 600,000 showed inhibited or delayed and feeble growth.

The Gram-positive organisms used for comparison, including sixteen strains of staphylococci, failed to grow at 1 to 500,000 dilution of the dye. In a few instances a slight growth developed along the edge of the slant, probably due to the dilution of the dye by the water of condensation.

The streptococcus-pneumococcus group, even the feebly growing strains, is, therefore, more resistant to the action of the violet dyes than other Gram-positive bacteria, growing at dilutions which still inhibit the other Gram-positive bacteria. This may be found of value in isolation from mixed material.

The above observations explain the irregular results in the streptococcus-pneumococcus group given in Churchman's protocols. Stowell, Hilliard and Schlesinger (4) found regular inhibition of nearly all their strains.

The influence of decolorization by sodium sulphite on the restraining action of gentian violet was very slight. In a concentration of 1 to 50,000 or 1 to 100,000 of the dye, there was no growth of either the staphylococcus or hay bacillus, when a dilution of 1 to 400,000 was reached, there was very feeble growth of the staphylococcus, but not of the hay bacillus.

While the above work was in progress, a further article by Churchman appeared (5). He found similar results using allied dyes. As he used only four strains for testing, and, as far as we can determine, only one dilution of the dye, he naturally found none of the variations in individual dyes, in which we were especially interested.

Summary

Gentian violet and allied anilin dyes have a similar influence on bacterial growth, dividing bacteria into two groups corresponding in general to their reaction to the Gram stain.

Among Gram-negative bacteria a strain is occasionally encountered which will not grow on violet agar, differentiating it from other members of the same species or variety.

The reaction is quantitative, although the quantitative character is more marked with some species than with others.

The streptococcus-pneumococcus group differs from other Gram-positive bacteria in their ability to grow in the presence of amounts of dye suffiffcient to inhibit the other species.

The dysentery bacillus group shows marked variation in the presence of dyes. In the case of fuchsin the variation approaches closely a specific difference between the dysentery and paradysentery groups. The variations of the latter groups with other dyes show no correlation with the common differential characteristics. A closer study might reveal variations in other characteristics which would parallel the different reactions to dyes. Decolorization with sodium sulphite robs the dyes of some of their inhibitive powers.

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DAHLIA AGAR AS A MEANS OF DIFFERENTIATION OF CHOLERA AND OTHER VIBRIOS

Charles Krumwiede, Jr., and Josephine S. Pratt

When in the fall of 1911 we were suddenly called upon to examine a large number of stools from suspected cholera carriers no agglutinating serum was obtainable. This was due to the fact that the necessity for cholera diagnosis had not arisen in New York City for over fifteen years. The second stool examined contained a vibrio, morphologically indistinguishable from true cholera. It was only after the culture isolated failed to produce indol that it could be excluded as a non-cholera type.

When the necessity for examining material for cholera vibrios arises only occasionally, and at intervals of years, it is evident that serum for agglutination may not be easily obtainable. It is in such circumstances that a simple culture method to differentiate cholera from other vibrios would be of the greatest value, especially, if we consider how frequently non-cholera vibrios may be present in stools. In one series of cases we found that 50 per cent. of the stools contained non-cholera vibrios.

Recently Signorelli has reported that cholera vibrios are capable of taking up the dye from agar containing dahlia, appearing in the decolorized mediums as violet colonies, whereas other vibrios do not decolorize the medium and form colorless colonies.

Signorelli believes that this reaction to dahlia agar is a practical means of differentiation between cholera and other vibros. In repeating Signorelli's work, we used cultures of cholera and other vibrios isolated from stools, as well as some laboratory strains of various vibrios.

Signorellie found that luxuriant, deeply colored colonies developed in agar containing 0.05 c.c. of a 1 per cent. watery solution of dahlia to every 10 c.c. of agar, that is a final dilution of 1-20,000 of the dye. We soon found that this concentration (using Grübler's Dahlia) exerted a marked restraining influence upon the growth of our strains. Because of this, we used higher dilutions, namely, 1-50,000, 1-75,000 and 1-100,000. Both slants and plates of ordinary thickness of the dahlia-agar were used. In the dilution of 1-100,000 the colonies were only slightly colored, but this dilution gave valuable information as to the inhibitory power of the dye. The strongest concentration likewise showed clearly the influence of the dye on the growth of the different vibrios, in many instances completely inhibiting growth. For inoculating the medium we used a millimeter loop of a fresh peptone water culture which was thoroughly shaken. If more than a millimeter loop was used, growth frequently took place, whereas, there was none with less heavy seeding. Heavy seeding, therefore, gave less reliable results, and conclusions in regards to the inhibition by the dye were less easy to determine. For controls, the same strains were planted on plain agar, using the same batch of medium as used with the die.

From our results given in the following tables, it will be seen that there is no special difference in the absorption of the dye by cholera and non-cholorea vibros. There is, however, a definite quantitative variation.

Culture	Indol	Growth on Agar	Incubation time in hours	On Dahlia Agar					
				1 : 100 000		1 : 75 000		1 : 50 000	
				Growth	Color	Growth	Color	Growth	Color
Chol. 1132	+	B	18	++++	± to +	+ to +++	+ to +++	±	++
			48	++++	+	++ to +++	++ to +++	+ to ++	+++ to ++++
Chol. 1165	+	D	18	+++	+	+ to +++	+ to ++	±	+
			48	++++	+	+++	++ to +++	++	+++
Chol. 387	+	C	18	+++ to ++++	± to +	+ to ++	± to ++	+ to ++	+ to ++
			48	++++	± " +	+++	++ to +++	++ to +++	++ to +++
Chol. 388	+	D	18	++++	± to +	++ to +++	± to ++	++ to +++	++ to +++
			48	++++	± " +	+++ to ++++	+ " ++	+++	+++
Chol. 390	+	B	18	+++	± to +	± to +	± to +	—	
			48	+++	± " +	+ " ++	+ " +++	— to +	++
Chol. 5054	+	B	18	+++ to ++++	± to +	± to +++	+ to ++	+ to ++	+ to ++
			48	++++	± " ++	+++ to ++++	++ to ++++	++	+++ to ++++
Chol. 41	+	B	18	++ to ++++	± to +	— to +	± to +++	— to ±	+ to +++
			48	++++	+ " ++	+ " ++	++ to +++	± " +	++ to +++
Chol. Prussia	+	B	18	+++ to ++++	+	++	+	— " +	+
			48	++++	+	++++	++ to +++	++	++
El Tor	+	B	18	++++	±	— to ±	—	—	
			48	++++	+	+	++ to +++	±	++
Vibrio 1162	—	B	18	+++ to ++++	±	++ to +++	± to ++	— to +	+ to ++
			48	++++	— to ±	++++	± " +++	+	++ to +++
Vibrio 101	—	A	18	++++	±	++++	+ to ++	++++	++
			48	++++	— to ±	++++	± " +	++++	± to +
Vibrio 105	—	A	18	++++	± to +	++++	— to ++	+++	++ to +++
			48	++++	— " +	++++	± " +++	++++	+ to ++
Vibrio 109	—	C	18	—		—		—	
			48	—		—		—	
Vibrio 111	—	D	18	—		—		—	
			48	—		—		—	
Vibrio 113	—	A	18	++++	±	++++	± to ++	++++	+ to ++
			48	++++	— to ±	++++	— " ++	++++	+

Growth on Agar Containing Dahlia—Continued

Culture	Indol	Growth on Agar	Incubation time in hours	On Dahlia Agar					
				1 : 100 000		1 : 75 000		1: 50 000	
				Growth	Color	Growth	Color	Growth	Color
Vibrio 115	—	C	18	++++	±	++ to ++++	+ to ++	± to ++++	+ to ++
			48	++++	±	+++ to ++++	+ " ++	+++ to ++++	++
Vibrio 117	+	A	18	+++ to ++++	±	+++ to ++++	+	+++ to ++++	++
			48	++++	± to +	++++	+	++++	+
Vibrio 119	—	B	18	++++	±	++++	+ to ++	+++	++ to ++++
			48	++++	— to +	++++	+ " ++	+++	++ " ++++
Vibrio 121	—	A	18	+++	± to +	++ to +++	++ to +++	±	?*)
			48	++++	± " +	+++	++ " +++	+	+++
Vibrio 123	—	A	18	++++	± to +	++ to ++++	+ to ++	+++	+
			48	++++	— " ±	++++	± " +	++++	±
Vibrio 125	—	A	18	++++	± to +	— to ++	+ to ++	— to ±	+
			48	++++	— " ±	± " ++	+ " ++	±	++
Vibrio Moltke	+	A	18	+++	± to +	— to +	± to ++	— to ±	?*)
			48	++++	±	+ " +++	+ " +++	± " +	++
Sp. Protea	—	B	18	± to ++	±	±	+	—	
			48	++ to +++	+	++	++ to +++	+	++ to +++
Sp. Schuylkilliensis	+	C	18	—		— to ±	?*)	— to ±	?*)
			48	— to +	++	— " ±	+++	— " ±	+++
Sp. tyrogena	—	C	18	— to ±	±	— to ±	±	—	
			48	± " +	+	— " +	± to ++	—	
Sp. Metchnikovii	+	C	18	±	?*)	— to ±		—	
			48	++	++	+	++ to +++	±	+++
Sp. phosphorensis	+	B	18	++++	± to ++	+++ to ++++	+ to ++	+++	+ to +++
			48	++++	+ " ++	++++	++ to +++	+++ to ++++	+++ to ++++
Sp. Milleri	—	B	18	++++	— to +	± to +++	± to +	+	±
			48	++++	± " ++	+++	++ to +++	+++	+++ to ++++

*) Insufficient Growth.

EXPLANATION OF TABLE:

Growth: ++++, as on agar without dahlia
± feeble growth
+, ++, +++, intermediate grades

Color: ++++, intense color
±, trace of color
+, ++, +++, intermediate shades

Growth on Agar: A, strains showing most vigorous growth
B, C and D, strains of decreasing vigor of growth

The amount of growth of individual strains is especially noted, for it bears in general an inverse relation to the color of the colonies. When a strain grows freely and is not inhibited by the dye, the color of the colonies is much less intense.

It was also noted that, as the growth increases, the intensity of the color lessens or even entirely disappears; thus it would seem as if the dye became diluted by being spread over the increased amount of growth. It is possible that rapid growth robs the dye of some of its oxygen thus reducing the color. The loss of color was especially evident in the large confluent colonies.

The readings were made after 18 and 48 hours incubation, and although, as noted in a few cases, the coloring was less intense, no specific differences were observed. When there was no inhibition by the stronger dilutions of the dye, the results were the same. In the table the indol production is given. Interested in the possibility of distinguishing cholera from non-cholera vibrios by simple rapid cultural tests, should serum not be available, we had previously studied the cultural characteristics of fifty non-cholera vibrios from stools.

Only seven of these vibrios produced indol; of the seven, four fermented glucose with gas production, two did not ferment, and only one fermented with the production of acid only, like true cholera. This last formed a very tough pellicle in peptone water and tenacious colonies on agar and because of these characteristics could be differentiated from cholera. Our work embraced over 3,000 examinations and the cultures isolated were tested for indol only during the early part of the work. It was soon evident, also, that most of the vibrios encountered in smears did not enrich in peptone water, while cholera does.* Exceptionally, however, it happens that in the first inoculation into peptone water, there will be limited development of cholera, while the second enrichment will show, in the upper layers of the medium, almost a pure culture.

Conclusions

Dahlia-agar is of no value for differentiating between cholera and other vibros, as no specific absorption of the dye occurs. When no immune serum is available, we must depend upon the ordinary cultural methods, which are sufficient to exclude nearly all vibrios, which are encountered in the routine examination of stools.

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2. Krumwiede, C., Jr., Pratt, J. S. and Grund, M., Cholera. (Journ. of Infect. Dis., Vol. 10, 1912, p. 134.)

* In our previous communication we said, "When mixed cultures containing vibrios are sub-inoculated in peptone water they do not show the same ability to enrich as shown by cholera." We were speaking of the vibrios as a group, not referring to individual strains. This has been misunderstood, and Ledingham and Arkwright, in "The Carrier Problem in Infectious Diseases," have quoted us as implying that individual strains do not enrich, in contraversion to the results quoted from Kolle. There are strains which enrich just as freely as cholera, and are culturally identical, and it would be advisable to limit the term cholera-like to these types, and use the term non-cholera vibrios for the others.

FUSIFORM BACILLI*

CHARLES KRUMWIEDE, JR., AND JOSEPHINE PRATT

1. ISOLATION AND CULTIVATION

Although an extensive literature exists on the fusiform bacillus, there are recorded no simple methods for the isolation and cultivation of these bacilli. The following methods were worked out in our endeavor to isolate a large number of strains for a comparative study of their cultural characteristics.

The isolation from shake and surface cultures was tried. These methods were found unsatisfactory. The possibility of success depends on an abundance of bacilli in the original material or the examination of smears from colonies till one might chance on a fusiform colony. The use of surface growths was not seriously considered, as we were endeavoring to dispense with the use of pyrogallic acid. Shake cultures in capillary tubes were then made so that the character of the colonies could be studied under the microscope. This was also found unsatisfactory. The cultivation in agar contained between two layers of glass was then tried. For this purpose the two halves of the petri dish were sterilized so that the bottom was placed in the inverted cover. The inoculated ascitic agar was poured into the cover and the bottom of the dish was laid on top of the agar while it was still fluid. A very few threaded colonies were noted and fishing from these gave fusiform bacilli. This gave us the basis for future work.

The original material is suspended in about 5 to 7 c. c. of ascitic fluid or horse serum, and successive dilutions made in a series of tubes containing the same amount. In making the dilutions a pipette is used carrying over about 0.1 to 0.2 c. c. to make the dilutions gradual. About 15 c. c. of fluid agar is then poured into the tubes and the mixture poured in the manner already described (Fig. 1). During incubation the plates should be covered with paper to limit air contamination of the rim of exposed agar, or the rim may be paraffined, which would also lessen the drying-out of the medium.



FIG. 1.

After incubation for forty-eight to seventy-two hours the upper layer of the dish is wrenched loose and the typical colonies searched for. These are characterized by threadlike outgrowths from one or both sides of

* This study had its inception in the interest of our associate, Dr. Matthias Nicoll, Jr., in the clinical conditions in which this organism is found. We are indebted to him for our material.

the colony. The general characteristics are shown in Figs 2 and 3. This type of colony develops only in the agar, not between the agar and the glass. More agar is employed than in the usual plate; otherwise a good seal between the two parts of the petri dish is not obtained. For this reason, in fishing, the needle must pierce the agar nearly vertically or the small colony is missed. One way is to bend the end of the wire so that it pierces the agar vertically, and with a circular motion break up the colony. Other bacteria give a similar colony but they are not common in the material containing fusiform bacilli. In two instances we isolated from similar colonies a motile anaerobic strepto-bacillus.

For cultivation and preservation of stock cultures we have found the most satisfactory medium to be a semisolid mixture of gelatin and agar. The constituents are as follows:

Agar	10 gms.	2 parts.
Gelatin	80 gms.	
Broth....2 per cent. peptone, no salt, 1,000 c. c.		
Horse serum or ascitic fluid		1 part.

Slightly alkaline to litmus is a satisfactory reaction.



FIG. 2.—Colonies, 22 hours old.

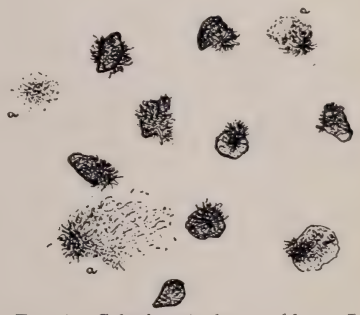


FIG. 3.—Colonies, 48 hours old. *a*, Surface or sub-surface colonies.

Inoculation is made by stab. The advantages over agar are that the puncture closes better after inoculation, the medium does not split up on drying, and subinoculation is easy, due to the softness of the medium. Aerobic contamination is promptly noted by growth in the upper layer of the medium and a motile contaminant, likewise, would be evidenced by diffusion of the growth.

Although the various strains differ in their ability to grow on simple media, the only sure method of cultivating all strains is in the presence of serum. Horse serum is better than ascitic fluid, possibly, because of the variations in the constitution of the latter. Freshly mixed media give much better results, probably because boiling the agar-gelatin medium drives off the contained oxygen.

As to the method of isolation, the ease with which strains may be isolated is evident from our results. Counting all the cases attempted,

including those tried while perfecting our methods, we have isolated 18 strains in 42 trials. In our last seven cases we isolated a fusiform seven times.

None of the observations given have any priority. In a subsequent examination of the literature we found that Ellermann gives a picture of a similar colony and several authors speak of the extensions from the colony, observations apparently made from pure cultures. No practical application of this fact was possible with the methods in use, should numerous colonies of other organisms develop. A priori we expected to find something distinctive in the colony, making this deduction from the morphology of the bacillus. The technic is given purely as a simple method requiring no apparatus and dispensing with cumbersome means of obtaining anaerobic conditions. As such it is the first simple practical method for isolation of these bacilli.

2. Cultural Characteristics

The strains isolated have come from varied sources and have been studied to determine whether they could be differentiated, one from the other, according to their cultural characteristics, and whether, if differentiation is possible, the grouping of the cultures has any relation to their source.

Although there is an extensive literature on the cultural characteristics of the fusiform bacillus, most of the reports deal with one or at most a few strains and are of little value for comparison and differentiation of strains. For this reason we shall report simply our own results.

Cultures Studied and Their Sources

1. Noma—recovering after salvarsan.
2. Ulceration of tongue from carious tooth.
3. Vincent's angina.
4. Discharging ear.
5. Discharging ear.
6. Pyorrhea.
7. Spongy bleeding gums.
8. Vincent's angina.
9. Vincent's angina.
10. Pyorrhea.
11. Vincent's angina.
12. Noma.
13. Carious tooth.
14. Vincent's angina.
15. Discharging ear.

Morphology.—The typical organism from the lesion is a double-pointed bacillus, containing one or more granules. The morphology is very variable in cultures, varying with the culture medium employed. On or in solid media the morphology is similar, though the forms are somewhat longer. In the water of condensation of agar slants or in fluid

media there is a tendency toward the development of filamentous forms, often forming tangled threadlike masses. In some of the filaments the granules may be absent. The typical organism is straight or somewhat bent. The filaments are at times sinuous and where granules are absent simulate a loosely wound spirochete. These are more common in fixed preparations. In fluid media, on or in solid media, when grown under identical conditions our strains showed no morphological characteristics that would be of value in differentiation of one strain from another. The only variation noted was the tendency of Nos. 2 and 3 to produce a greater proportion of short forms.

Motility.—Repeated examinations, in hanging-drop preparations and with dark field illumination, of original material and of bacilli from the most varied types of culture media, have failed to show any evidence of motility.

Gram stain.—None of the strains retained the dye.

Colony morphology.—The characteristic colony, as already described, develops only in the depths of agar.

General Characteristics of growth.—As we have stated, serum or blood is necessary for satisfactory cultivation of the strains. We have referred to the use of a semi-solid agar-gelatin mixture which facilitated the transfer of stab cultures. We have found that one-half of 1 per cent. agar with the addition of one-third of its volume of serum gives satisfactory results and is much easier to prepare. After isolation, growth may take place in serum-free media for several generations, but for continued vigorous development and surety of transfer a serum medium has been found necessary.

On or in agar the growth of all the strains was the same. In stab or shake culture a puff-ball appearance is present, as one would expect from the appearance of the colony. On serum or blood agar the growth is less characteristic, consisting of a delicate greyish-white non-adherent layer becoming more opaque with longer incubation and developing a heaped-up appearance.

In broth a flocculent growth occurs at the bottom of the tube, leaving the upper portion clear. The simplest method for cultivation in serum broth is to sterilize the broth covered with a layer of albolene and add to each tube one-third to one-fifth the volume of heated serum. With this method the results are as good as with more complex means of producing anaerobosis.

Indol production.—All strains produced a large amount of indol.

Odor.—All cultures had a similar disagreeable odor.

Viability.—After cultivation for several generations the strains remain viable for a long period. Some strains were viable 67 days when preserved on ice. In the incubator three or four strains were viable up to six months.

The viability of freshly isolated strains was not tested. Although various batches of media are unequally adapted for their cultivation, they can be easily kept alive in semi-solid serum media. Some of our strains are now in the fiftieth to sixtieth generation.

SUGAR FERMENTATIONS

No.	Dextrose	Galactose	Levulose	Arabinose	Raffinose	Lactose	Saccharose	Dextrin	Maltose	Mannite	Mannose	Dulcite	Rhamnose	Inulin	Glycerin
1	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
2	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
3	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
4	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
5	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
6	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
7	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
8	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
9	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
10	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
11	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
12	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
13	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
14	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
15	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—

No gas formation was noted in any of the tubes. This fact, as well as the differences in morphology, differentiates the fusiform bacillus from the bacillus necrosis which produces gas from dextrose.

Method.—To a sugar-free semi-solid agar sufficient sugar solution was added to bring the content to 1 per cent., and finally the necessary serum. Litmus was used as an indicator, the color being restored by softening the agar and pouring into a petri-dish. Nearly all the results were later checked by titration which gives sharper readings. For sterilization the sugar solutions were usually heated 10 minutes. In the case of maltose, sterilization was accomplished by filtration. In a few series the inoculated tubes showed a slight increase of acidity over the controls. As the non-fermenting controls gave the same change, this was referable to slight cleavage of the sugar and the formation of fermentable monosaccharides. This deduction was further proved by the fact that the positive controls gave a marked increase of acidity comparable with the acid production of some fusiform strains with saccharose.

Relation of the fusiform bacillus to the spirochetes from the same source.—In the smears made from the original material, loosely wound spirochetal forms comparable with those found in fusiform cultures are present. The typical spirochetal forms seen in the original material have never been encountered in pure cultures of the bacillus. The cultural forms simulating spirochetes have been found in fixed preparation. Repeated examinations with dark field illumination have revealed no forms that could in any way be compared with those found in original material. Furthermore the spirochetes in the original material, morphologically at least, may be of two or possibly three varieties. These can be kept alive in mixed culture in coagulated horse serum, the fusiform bacilli dying out on transfer. When pure cultures of fusiform bacilli are planted in

this medium no change of morphology takes place. The motility of the spirochete and the lack of motility of the fusiform bacillus form the strongest evidence against their identity. The observations of cultural forms have embraced the growth on the most varied media and at every stage of incubation.

Summary

The fusiform bacilli isolated by us fall in two groups, one group fermenting saccharose, the other not.

There is no relation between these groups and the source of the culture.

The fusiform bacillus and the spirochetes accompanying it are separate and distinct organisms.

THE RELATIVE IMPORTANCE OF THE BOVINE AND HUMAN TYPES OF TUBERCLE BACILLI IN THE DIFFERENT FORMS OF TUBERCULOSIS

(Final Summary of Cases Investigated)

WILLIAM H. PARK AND CHARLES KRUMWIEDE, JR.

Although we have examined no further cases since the publication of our last report,[1] there have been numerous reports from abroad.

Outside of the tables we have already published there is no complete and simple summary of the cases investigated. We have, therefore, brought these tables up to date and present them for their statistical value. Technical details are not touched upon. These have already been fully discussed.

The method of classification used in preparing the tables is given in our first report.[2]

In the following tables are included the recent reports of Weber, Steffenhagen, Dieterlen, Lindeman, Ungerman, Kossel, and Eber. Jansco and Elfer have examined a series of cases, but these could not be included, as the individual details and ages are not given.

The new cases given in the final report of the Royal Commission are also included, with the exception of Virus H. 16 "J. H." This case was an infection of the kneejoint. The commission consider this a mixed infection of the bovine and human types. The proof of this does not seem to us at all convincing.

The case of De Jong-Stuurman, given in the former tables as a doubtful case of pulmonary tuberculosis due to the bovine bacillus, is included as other like cases have been found.

Where the figures under children are divided by a vertical ruling those to the left of the line are the cases dying of tuberculosis, and those to the right are latent cases or those discovered at autopsy or operation.

TABULATION OF CASES REPORTED BY OTHERS

Diagnosis	Adults 16 Years and Over		Children 5 to 16 Years		Children Under 5 Years	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary tuberculosis	497	3	5 | 1	—	25 | 3	1 | —
Tuberculous adenitis, axillary	2	—	—	—	2	—
Tuberculous adenitis, cervical	27	1	17	14	9	11
Abdominal tuberculosis	15	4	1 | 6	3 | 5	3 | 6	7 | 4
Generalized tuberculosis, alimentary origin	6	1	3 | —	4 | —	15 | 1	13 | —
Generalized tuberculosis	27	—	3 | 1	1 | —	46 | 10	2 | 1
Generalized tuberculosis, includ- meninges, alimentary origin	—	—	1	—	5	10
Generalized tuberculosis, including meninges	4	—	10	—	51	—
Tubercular meningitis	—	—	1	—	2	2
Tuberculosis of bones and joints	31	1	31	3	20	—
Genito-urinary tuberculosis	16	—	1	—	—	—
Tuberculosis of skin	9	3	4	6	2	—
Miscellaneous cases—						
Tuberculosis of tonsils	—	—	—	1	—	—
Tuberculosis of mouth and cervical nodes	—	1	—	—	—	—
Tuberculous sinus or abscess	1	—	—	—	—	—
Sepsis, latent bacilli	—	—	—	—	1	—
Totals	635	14	85	37	201	51

Mixed or double infections:—	10 cases:	
Pulmonary tuberculosis...	20 years	Sputum, human and bovine types
	27 years	Sputum, human and bovine types
Abdominal tuberculosis...	70 years	Mesenteric nodes, human type Retroperitoneal, human and bovine type
Generalized tuberculosis...	5½ years	Spleen, human type Mesenteric node, bovine type
Alimentary origin	18 years	Lung, culture not obtained Mesenteric nodes, human and bovine types
	30 years	Bronchial node, human type Mesenteric nodes, human and bovine types
Generalized tuberculosis...	9 months	Bronchial nodes, bovine type Mesenteric nodes, human type
Generalized tuberculosis, including meninges... Alimentary origin	4 years	Meninges, human type Bronchial nodes, human type Mesenteric nodes, bovine type
Generalized tuberculosis, including meninges.....	4 years	Bronchial nodes, Spleen, injected together, bovine and human types
	4 7/12 years	Meninges, human type Lung, human type Mesenteric nodes, human type Bronchial nodes, human and bovine types

TOTAL CASES—1,033

Adding to this table our series of four hundred and seventy-eight cases (see table[3]), gives the following complete tabulation:

COMBINED TABULATION OF CASES REPORTED AND OWN SERIES OF CASES

Diagnosis	Adults 16 Years and Over		Children 5 to 16 Years		Children Under 5 Years	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary tuberculosis	778	3	14	—	35	1
Tuberculous adenitis, axillary or inguinal	3	—	4	—	2	—
Tuberculous adenitis, cervical	36	1	36	22	15	24
Abdominal tuberculosis	16	4	8	9	10	14
Generalized tuberculosis, alimentary origin	6	1	3	4	17	15
Generalized tuberculosis	29	—	5	1	74	7
Generalized tuberculosis, including meninges, alimentary origin	—	—	1	—	5	10
Generalized tuberculosis, including meninges	5	—	10	—	76	1
Tubercular meningitis	1	—	3	—	28	4
Tuberculosis of bones and joints	32	1	41	3	27	—
Genito-urinary tuberculosis	22	1	2	—	—	—
Tuberculosis of skin	10	3	4	6	2	—
Miscellaneous cases—						
Tuberculosis of tonsils	—	—	—	1	—	—
Tuberculosis of mouth and cervical nodes	—	1	—	—	—	—
Tuberculous sinus or abscess	2	—	—	—	—	—
Sepsis, latent bacilli	—	—	—	—	1	—
Totals	940	15	131	46	292	76

Mixed or double infections, 11 cases

TOTAL CASES—1,511.

Taking these cases and estimating the percentages of bovine infection gives the following table. The percentages in the cases of alimentary

infection are given separately, as many of them were selected and bear too great a proportion to the total.

PERCENTAGE INCIDENCE OF BOVINE INFECTION

Diagnosis	Adults 16 Years and Over	Children 5 to 16 Years	Children Under 5 Years
Pulmonary tuberculosis	.4%	0%	2.8%
Tuberculous adenitis, cervical	2.7%	38%	61%
Abdominal tuberculosis	20%	53%	58%
Generalized tuberculosis, alimentary origin	14%	57%	47%
Generalized tuberculosis	0%	16%	8.6%
Generalized tuberculosis including meninges, alimentary origin	0%	0%	66%
Tubercular meningitis (with or without generalized lesions other than preceding)	0%	0%	4.6%
Tuberculosis of bones and joints	3.3%	6.8%	0%
Tuberculosis of skin	23%	60%	0%

As is evident from the table summarizing the total cases reported, many of those in children had slight or latent infections, found on their death from other causes. The percentages deduced, therefore, only give the incidence of infection, nothing more.

Omitting all but the fatal cases of tuberculosis from this series and adding the ninety-one fatal that we investigated gives the following table. It is evident that in some instances the figures are too small to deduce final percentages.

TOTAL CASES IN CHILDREN. REPORTED AND OWN CASES*

Diagnosis	Children 5 to 16 Years		Children Under 5 Years	
	Human	Bovine	Human	Bovine
Abdominal tuberculosis	1	3	3	10
Generalized tuberculosis, alimentary origin	3	4	16	14
Generalized tuberculosis	4	1	64	5
Tubercular meningitis, secondary to tuberculosis of alimentary origin	1	..	5	10
Tubercular meningitis (other than preceding)	13	..	104	6
Totals	22	8	192	45

* Exclusive of double or mixed infections.

TOTAL FATAL CASES—267.

Fatality Percentages of Bovine Infections

Diagnosis	Children 5 to 16 Years	Children Under 5 Years
Abdominal tuberculosis	75%	77%
Generalized tuberculosis, alimentary origin	57%	46%
Generalized tuberculosis	20%	7.2%
Tubercular meningitis, secondary to tuberculosis of alimentary origin	0%	66%
Tubercular meningitis (other than preceding)	0%	5.4%
Total percentages	26%	18%

The percentages deduced from the total figures cannot be applied directly. Due to selection by many investigators, the number of cases of alimentary infection, which as the table shows are very likely to be bovine infections, is disproportionate. It must not be forgotten that the earlier investigators selected their cases in an endeavor to prove that man could be infected with the bovine bacillus.

In our own series of non-selected fatal cases under five years of age bovine infection constituted twelve and one-half per cent. We had nine cases under six years of age, who were exclusively cow's milk fed, from a foundling asylum. Of these, five were bovine infections. If the fatal cases in this series are deducted from the total cases, the bovine infections comprise about ten per cent. In a non-selected series of fatal cases from the Babies Hospital six and one-third per cent were due to bovine infection.

Where other investigators have reported non-selected cases the percentages come close to these figures.

On the whole, bovine infection causes somewhat less than ten per cent of the total deaths in young children.

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TESTS OF HEMOGLOBINOPHILIC BACILLI FROM CONJUNCTIVITIS IN REGARD TO THEIR VIRULENCE AND THEIR AGGLUTINATING PROPERTIES

By Olga R. Povitzky

These tests were undertaken at the suggestion of Dr. A. W. Williams of the Research Laboratory, in connection with the work on trachoma (see page 159). The cultures worked with were isolated, some from cases diagnosed "trachoma," acute conjunctivitis or border line cases, in the various clinics for Infectious Eye Diseases of the Health Department; some from deportable "trachoma" cases on Ellis Island; some from an epidemic conjunctivitis in Port Jefferson.

Virulence Tests.

Some of the freshly isolated strains from the eye are quite virulent for the guinea pig on intra-peritoneal inoculation. Thus strains 636, 29, 8—the former isolated from a case of "trachoma" (deported eventually) on Ellis Island, the other two from a recent epidemic of conjunctivitis in Port Jefferson, L. I.—killed a 200 gram guinea pig in 20 hours in one small slant culture (see Table I).

TABLE I

Virulence for Guinea Pigs of Hemoglobinophilic Bacilli Intraperitoneal Inoculation Recently Isolated from Cases Diagnosed Trachoma

Date	Weight	Strain	Amount in Small Slants	Course	Autopsy
2/29/12	220 gms.	636	4	Died in night	Pure cultures of bacilli from heart's blood and peritoneal fluid
2/29/12	230 gms.	636	2	Sick, recovered	
2/29/12	210 gms.	636	1	Died in 20 hours	Same as first
3/26/13	200 gms.	8	1	Died in 18 hours	Same as first
3/26/13	200 gms.	8	2	Died in 18 hours	Same as first
3/26/13	210 gms.	8	1½	Very sick, recovered	
3/26/13	200 gms.	29	1	Died in 24 hours	Same as first

When the cultures are carried on for some time on artificial medium (blood-veal-agar) their virulence is gradually diminished so that at the end of a year not less a dose than 4Tb* tube slants or 2 potato† tube slants is fatal to a 200 gram guinea pig (as shown in Table II).

* ¾ x 6 inches.
† 1 x 6 inches.

TABLE II

VIRULENCE OF CULTURES AFTER BEING GROWN FOR SEVERAL MONTHS ON ARTIFICIAL CULTURE MEDIA

Date	Weight	Strain	Amount in Potato Tubes	Course	Autopsy
3/26/13	220	636	3 cult.	Died 3d day	Pure culture obtained from heart's blood and peritoneal fluid
3/26/13	220	636	1	Recovered	
10/10/12	200	186	3	Died next day	Same as first
10/10/12	200	186	2	Sick, recovered	
10/10/12	210	186	1	Well	

Delius-Kolle (1) in experimenting with the freshly isolated influenza bacilli from influenza cases, influenza-pneumonia and influenza-phthisis found that guinea pigs succumbed to a 2/4 slant isolated from the first disease, 1/20 to 1/10 slant from the second disease and 1/2 to 3/4 slant from the third. The virulence was much diminished after 4 weeks. Thus the strain which killed in 1/20-1/10-slant required 1/4-1/3 for the fatal dose. The same strain after one year did not kill guinea pigs in one slant doses.

The lost virulence may be restored gradually by a number of passages through the pig. Thus strain 186 (one of the first isolated strains from a case of "trachoma"), was tried for virulence one year after isolation and was found to be fatal to the guinea pig only in 3 potato-tube doses.

After a number of consecutive passages through the pig it gradually regained its virulence till after the 9th generation in pigs it killed a 200 gram guinea pig with 1/2 small slant in 20 hours and after the 13th generation 1/10 of a slant was fatal to the 200 gram guinea pig in 18 hours (see Table III), while the original strain grown on the stock horse blood-agar continued to remain only slightly virulent (Table III).

In order to maintain the high virulence it is imperative to resort to immediate passage through the pig, after isolation of the organism otherwise its virulence may be impaired even in one week.

Cantani (2) also speaks of the rapid loss of virulence in cultures isolated from influenza. He had to resort constantly to animal passages to keep the strains virulent.

The virulence of the organism varies very much with the size and age of the pig. The smaller and younger the pig the less resistant it is to the pathogenic action of the hemoglobinophilic organism. A 300 or 400 gram pig can stand quite a large dose of even a very virulent strain. The blood contents of the medium is also of some importance. A culture grown on 1-100 blood is less vigorous than that grown on 1-5 blood. Recently we found that a more vigorous growth is obtained on coagulated

blood medium. This medium is obtained by adding blood to agar (1 to 10 to 1 to 500) at 90° C.

Cultures grown on blood agar and killed at 60° for one and half hour did not kill the pig while controls of the living bacteria in the same dose killed them the same night or next morning.

Experiments with sterilized blood broth cultures were very uncertain. Delius & Kolle (1) who experimented with influenza bacillus from epidemic influenza found contrary to our results, with bacilli from "trachoma" cases that the sterilized agar cultures were quite poisonous to the guinea pig. The broth-blood-cultures were not more poisonous than the medium itself.

Rabbits seem to be irregularly susceptible to these hemoglobinophilic bacilli even when recently isolated from the human eye and appear even less susceptible to the organism which passed through many generations of pig. Thus strain 186 after the 13th passage killed the guinea pig in 1/10 slant but did not make the rabbit even ill with 2 bottles (100 c. c.) of a very vigorous growth.

Some rabbits were inoculated intravenously with surface cultures on blood agar in one pint bottles of recently isolated strains from cases of conjunctivitis without any ill effects. Recently a strain (No. 8) was found which was isolated from a case diagnosed as "trachoma" in Port Jefferson and when grown on coagulated blood proved to be quite virulent for the rabbit by intravenous injection (Table IV).

Pfeiffer (3) and Cantani (2) found the rabbit very susceptible to the poison of influenza bacilli isolated from cases of influenza. Centani had to resort to guinea pigs for immunization tests as the rabbits stood very badly inoculations with influenza bacilli.

The autopsy of guinea pigs and rabbits which die of this hemoglobinophilic organism, reveal intense congestion of the organs; some pericardial and peritoneal exudate. The smear from peritoneal fluid shows a great multiplication of the organism, and a pure culture of hemoglobinophilic bacillus is invariably obtained from planting the hearts' blood as well as the peritoneal fluid.

TABLE III

VIRULENCE OF LAST CULTURE AFTER CONSECUTIVE PASSAGES THROUGH GUINEA PIG*—INTRAPERITONEAL INOCULATION

Date	Weight	Strain	Amount	Course	Autopsy
12/5/13	210	186	1 pot. tube	Died next morning	Pure culture from heart's blood and peritoneal fluid
12/5/13	200	186	1 tb. tube	Recovered	
1/5/13	200	186	½ tb.	Died	Same as first
1/5/13	200	186	1 tb. tube	Died in 20 hours	
2/7/13	210	186	1/10	Died same night	Same as first

*After 5th, 9th and 13th passage through the pig.

TABLE IV

VIRULENCE FOR RABBITS OF HEMOGLOBINOPHILIC BACILLI FROM "TRACHOMA" —48 HOURS' CULTURES GROWN ON BLOOD AGAR (BLOOD ADDED AT 50° IN THE FIRST 4 CULTURES, AND AT 90° IN THE LAST 3)

Date	Weight	Strain	Route	Amount	Course
11/28/11	1,600	357	Intra-venous	1 qt. bot. Vig. growth	Recovered
11/28/11	1,600	169	Intra-venous	1 qt. bot. Vig. growth	Recovered
11/28/11	1,500	601	Intra-venous	1 qt. bot. Vig. growth	Recovered
11/28/11	1,500	186	Intra-perito	5 100 c. c. bot.	Recovered
11/28/11	1,550	186	Intra-perito	10 100 c. c. bot.	Recovered
7/9/13	1,650	186	Intra-venous	4 bot. 100 c. c.	Died next day Pure culture recovered
7/9/13	1,590	8	Intra-venous	3½ c. 100 c. c. bot.	Died same evening
7/23/13	1,550	8	Intra-perito	3 bot.	Recovered, not sick
7/23/13	1,560	8	Intra-venous	2 bot.	Found dead in the morning
7/23/13	1,550	8	Intra-perito	4 bot.	Recovered

SUMMARY

These strains of hemoglobinophilic bacilli isolated from all grades of conjunctivitis seem to show virulence characteristics similar on the whole to those described for the influenza bacilli, *i. e.*, guinea pigs and rabbits are susceptible in a variable degree to freshly isolated cultures. 2—Cultures lose virulence rapidly but may be made again virulent for the guinea pig after successive passage of the organism through the animal. Such a guinea pig strain is not virulent for the rabbit. 3—On coagulated blood-agar medium, these strains grow very vigorously and measured by tube growth are more virulent than cultures grown on ordinary blood-agar medium (blood added to agar at 50°).

AGGLUTINATION TESTS

A number of rabbits were immunized against different strains of hemoglobinophilic organisms isolated from cases of conjunctivitis (mostly diagnosed as trachoma), and two strains of the influenza bacillus, BI., BI., given to us by Dr. Wollstein, isolated respectively from cases of meningitis and bronch-pneumonia. The animals were inoculated every 6-7 days with living cultures, beginning with ½ or ¼ surface growth of a 100 c. c. bottle gradually increasing the dose. The last dose was usually from

10-12 100 c. c. bottles of the organism. The injections were intraperitoneal and the rabbits were bled before inoculation and on the 9th day after the last inoculation for agglutination tests (see Table V). A great many strains were self-agglutinating and the sera of rabbits immunized against such strains could not be tested with the homologous strains. The self agglutinating property of some strains was obviated by using distilled water instead of salt for emulsions and dilutions of sera. Rabbits are somewhat variable in their agglutinating powers even when immunized with the same strains. Thus two rabbits were inoculated with 636 (from a case of "trachoma" on Ellis Island) one of which received 17-100 c. c. bottles in 7 weeks the other 22½-100 c. c. bottles in the same length of time. The first rabbit showed agglutination up to 1-1600 very strongly—the reaction appearing after one hour in the incubator, while the second showed agglutination only up to 1-800—the reaction not being fully established till next morning after being left in the ice box over night.

TABLE V

RABBITS IMMUNIZED WITH HEMOGLOBINOPHILIC BACILLI, BY INTRAPERITONEAL INOCULATIONS

Strain		Amount of Culture 100 c. c. Bot.	Length of Time of Inoc.	Time Between Bleeding and Last Inoc.	Agglut. Test with Hemologous Strains				
					1–40	1–400	1–800	1–1600	1–2400
1912	636	17	7 wks.	8 days	++	++	++	+	±
1913	636[1]	36	7 wks.	9 days	++	++	++	+	±
1912	213	26½	8 wks.	10 days	+	±	−	−	−
1912	416	27½	8 wks.	10 days	++	++	±	±	±
1912	281	27½	8 wks.	10 days	±	−	−	−	−
1912	186	24	6 wks.	10 days	+	±	−	−	−
1913	186	36	7 wks.	9 days	++	+	±	−	−
1912	B. I.[2]	24½	6 wks.	10 days	++	++	±	±	+ =
1912	B. I.1	36	7 wks.	9 days	++	++	±	±	−
1912	B. I.2	24½	6 wks.	10 days	−	−	−	−	−

(1)Strain 636 a year later became slightly self-agglutinating.

(2)B. I.1 was from meningitis. B. I.2 from broncho-pneumonia; all of the others were from comjunctivitis.

(3)Normal sera of rabbits before inoculation were either negative or like the control culture in salt. ± or ±. The latter control was also employed in Table VI.

Cross agglutination tests showed agglutinins common to the group in a great many strains. In testing about 30 strains and repeating the experiments over and over again—two strains were found to be very closely related in their agglutinating reaction (see Table VI).

TABLE VI

CROSS AGGLUTINATIONS OF HEMOGLOBINOPHILIC BACILLI WITH HETEROGENOUS SERA

Serum	Strain	Cross Agglutinations				
		1–40	1–400	1–800	1–1600	1–2400
636	336[1]	+	+	+	+	±
636	2,269	−	−	−	−	−
636	283	+	±	−	−	−
636	405	+	±	−	−	−
636	415	+	−	−	−	−
636	B. I.	−	−	−	−	−
636	186	+	+ =	−	−	−
636	213	+	±	−	−	−
636	281[2]	+	±	−	−	−
186	636[3]	±	±	−	−	−
186	B. I.	±	+ =	−	−	−
186	336[1]	−	−	−	−	−
186	213	−	−	−	−	−
416	B. I.[1]	−	−	−	−	−
416	636	±	±	−	−	−
416	336	−	−	−	−	−
416	213	−	−	−	−	−
B. I.	B. I.[2]	−	−	−	−	−
B. I.	186	±	−	−	−	−
B. I.	636	±	±	±	−	−
281	B. I.	±	±	±	−	−
281	213	−	−	−	−	−
213	336	+	−	−	−	−

(1)Strain 636 and strain 336 seem to be related though the fact that the former agglutinates slightly in 1 to 400 with serum of 186 strain while the latter does not, shows that they are not identical.

(2)No. 281 homologus sera aggl. in 1–40.

(3)No. 636 serum aggl. 186 in 1–40, 1–400.

Cantani (2) who immunized guinea pigs against influenza bacillus used for the first inoculation sterilized cultures and for subsequent inoculation living cultures. The guinea pig showed agglutination up to 1-500. He was less fortunate with rabbits as they were too susceptible to the influenza poison. Wollstein (4) immunized rabbits with influenza

strains designated "pneumonia" "tuberculosis" "adult influenza" and "eye" until their sera agglutinated the homologous organisms in dilution 1-400. The other strains were agglutinated by such sera up to 1-300 or 1-350 showing that agglutinins were common to the group. In repeating the experiments of the year before the author found that the rabbits developed agglutinins much more slowly than the old ones had done.

She did not get any agglutination reaction with the blood of a number of patients suffering with influenzal infection; only two patients showed agglutinations in 1-20 to 1-40 dilution.

Vagedes (5) examined the sera of 27 influenza patients, 8 of these gave positive reactions, 5 gave agglutination up to 1-50, 3 up to 1-100 on 3d, 8th, 11th day of the disease; 3 cases showed agglutination 3 weeks after the injection and one case 5 weeks later. Zichtner (6) used the same technique as Vagedes, except that he employed .85 per cent. salt solution instead of .4 per cent., but his results were far from convincing as he found most of the influenza strains self agglutinating.

A Macaccus was inoculated with strain 337 in the left eye every 4-5 days for 3 weeks and showed slight reaction without manifesting the organism in the smear or culture. Two weeks later the monkey was again inoculated with the freshly isolated strain 636 after which the eye showed intense reaction, a hemoglobinophilic organism being recovered from the culture of the infected eye. The uncertainty as to whether the infection was caused by the previous strain 337 or recent one 636 was cleared by the agglutination test. The serum of the rabbit immunized against strain 636 agglutinated this organism from the monkey's eye to 1-1600—the reaction appearing in one hour after being in the incubator, while this same serum did not agglutinate 337 in any dilution. Rabbits inoculated intraperitoneally with polyvalent influenza vaccine have not yet shown agglutination reactions.

SUMMARY

1. These studies on agglutination show that hemoglobinophilic bacilli from the eye are similar to hemoglobinophilic bacilli from other sources in their power to produce agglutinins.

2. Rabbits vary in their power to produce agglutinins when immunized with different strains of hemoglobinophilic bacilli isolated from all grades of conjuctivitis.

3. A great many strains of these bacilli are self agglutinating. Some of the latter may be made to lose this property by using distilled water for the diluent instead of salt.

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THE EFFECT OF VARIATIONS IN MEDIA ON GONOCOCCUS ANTIGENS

Anna I. van Saun

A series of experiments was undertaken in the autumn of 1912 and early part of 1913 to determine, if possible, the reason for the increasing anticomplementary quality and lack of antigenic properties of the gonococcus antigens used in the complement-fixation test for gonorrhea.

It was thought that the trouble was largely with the medium used for stock cultures, so three different media of varying reactions were selected for the experiments and duplicate sets of transplants kept thereon. The media used were neutral, glucose-ascitic agar, veal agar 0.5 per cent. acid, veal agar 0.5 per cent. acid with the addition of 5 per cent. glycerine, and veal agar neutral to phenolphthalein. The stock cultures were transplanted every other day, and the antigens made up once a week. Ten of the twelve strains of gonococcus isolated by Dr. John C. Torrey of the Loomis Laboratory were used in this work.

At the beginning of the experiments the stock cultures were kept on neutral, glucose-ascitic agar in an effort to restore their antigenic properties. These transplants had to be given up for a time owing to the difficulty experienced in procuring large amounts of ascitic fluid, and but four antigens were made from cultures grown on this medium. Of these one was poor, two were fair, and one was good possessing no anticomplementary qualities.

Eight antigens were made from stock cultures kept on veal agar with a reaction of 0.5 per cent. acid. The growth on this medium was almost always good, and only two of the antigens were poor. Three were fair, and three were good, although one of these was slightly anticomplementary. These antigens, however, were still unstable, sometimes deteriorating over night.

Stock cultures kept on veal agar with a reaction 0.5 per cent. acid and the addition of 5 per cent. glycerine produced a good growth part of the time, although the cultures were not as viable as those grown on other media. Two of the antigens produced from this stock were poor, one was fair, and three were good, although one was slightly anticomplementary. They were all more or less unstable and, as the cultures grew so poorly, the transplants were discontinued.

Neutral veal agar was the last medium tried. On it the growth was uniformly good and recently very good. None of the seven antigens made from stock grown on this medium were poor, four were fair, and three were good, though two of these were slightly anticomplementary. They were also more stable, although sufficient time has not elapsed to enable us to judge fairly as to their stability in comparison with former antigens made some time ago, and which were kept for six months.

In conclusion it may be said that as far as we have gone in our experiments, which are by no means completed, there seems more to be said in favor of neutral veal agar as a stock medium than of any of the

others tried. When this medium is made from four or five weeks old *veal*, an increase in the growth of the cultures and also in their antigenic properties has been noticed. The medium should not be more than one day old before being used, either for stock or antigens. Agar which is in the least dry is useless for gonococcus cultures.

The growth of the cultures appears to be of importance in the production of good antigen, although good antigen has occasionally been made from cultures which grew poorly. A good growth does not, however, always mean a good antigen. Out of the twenty-five antigens designated in this report eight good ones were produced from cultures which grew well, six antigens were only fair and two were poor. Four fairly good antigens were made from cultures which had only a fair growth and only one good antigen was obtained from cultures which grew poorly.

In the accompanying table the term "poor" in the column for antigen titrations refers to antigens which cannot be used, as they have no range. "Fair" indicates an antigen which is usable, but which has not much range, and possesses anticomplementary properties. "Good" refers to antigens with a wide range. A good antigen may be slightly anticomplementary, but this does not interfere with its usefulness in the test.

Polyvalent Gonococcus Antigen	Stock Medium	Titre of Medium	Growth	First Heating at	Second Heating at	Result of Antigen Titration
1	Ascitic	Neutral	Poor	56° C.	56° C.	Poor
2	"	"	Poor	"	"	Good
3	Salt veal agar	0.5% Acid	Good	"	"	Good
4	"	"	Poor	"	"	Poor
5	"	"	Fair	"	"	Fair
6	"	"	Good	"	80° C.	Fair
7	"	"	Very good	"	"	Fair
8	"	"	Good	"	"	Good Sl. Ac.
9	"	"	Good	"	"	Good
10	"	"	Good	"	"	Poor
11	Glyc. 5% veal.	"	Fair	"	"	Fair
12	"	"	Very good	"	"	Poor
13	"	"	Very good	"	"	Good Sl. Ac.
14	"	"	Very good	"	"	Good Ac.
15	"	"	Fair	"	"	Poor
16	"	"	Fair	"	"	Good Sl. Ac.
17	Salt veal agar	Neutral	Very good	"	"	Good
18	"	"	Good	"	"	Fair
19	Ascitic	"	Fair	"	"	Fair
20	Salt veal agar	"	Fair	"	"	Fair
21	"	"	Good	"	"	Fair
22	Ascitic	"	Good	"	"	Fair
23	Salt veal agar	"	Good	"	"	Fair
24	"	"	Very good	"	"	Good Sl. Ac.
25	"	"	Very good	"	"	Good Sl. Ac.

Sl. Ac.—Slightly anticomplementary.

The technique used at present for making the antigen is as follows:

Twenty-four hour cultures on the stock medium neutral salt veal agar are transferred to potato tubes of neutral salt-free veal agar and incubated for twenty-four hours. *All* the growth on these tubes is transferred by means of sterile cotton swabs to Blake bottles containing neutral salt-free veal agar, and these are incubated for twenty-four hours. This growth is washed off with neutral sterile distilled water, ten c. c. to a bottle, and the emulsion autolyzed at 56° C. for two hours in the water bath. It is then put through a Buchner funnel, and this filtrate through a Berkefeld filter. Finally it is heated to 80° C. for one hour in the water bath, and then to 56° C. for one-half hour on two successive days. It may be put up in small bottles with a thin layer of paraffin coating the surface of the liquid, and is ready for use.

A PRELIMINARY NOTE ON SPIROCHAETA PALLIDA AND LIVING TISSUE CELLS IN VITRO

Edna Steinhardt

Having successfully applied Harrison's method of tissue growing *in vitro* to the cultivation of the virus of vaccinia,* we have here tried the same method with the *Spirochaeta pallida*. In the present experiment the organisms used were from a culture obtained from Dr. Noguchi. He has kept it growing for some length of time and states that it has lost most of its virulence.

Hanging drop preparations were made of small pieces of the testes of the rabbits inoculated with spirochaetes to which rabbit plasma was added. The preparations were incubated at 37 deg. C. (98.0 F.). Before incubation preparations examined in the dark field did not show spirochaetes nor did those examined during the first few days of incubation. Evidently very few spirochaetes were inoculated. The cells of the testes, however, showed a good growth.

After incubation for twenty-five days examination with the dark field showed many organisms. These were extremely motile. Short forms found strongly suggested multiplication of the organism and not merely a survival of the original inoculation. The early negative examinations would also suggest a multiplication.

The organisms were inoculated into the testes of a rabbit and the virulence will be reported later. It seems possible by this method of growing avirulent cultures with living cells and transferring to fresh cells to restore their pathogenicity. This would be in line with the increase of virulence found in bacteria submitted to the action of immune serum. Further experiments are now being made on this direction and also on the direct inoculation of spirochaetes from syphilitic lesions.

* Steinhardt, Israeli, and Lambert: Jour. Infect. Dis., 1913, xiii, 294.

ON THE PRESENCE OF DIPHTHERIA AND DIPHTHERIA-LIKE ORGANISMS IN SCARLET FEVER PATIENTS

HARRIET L. WILCOX AND MARIAN S. TAYLOR

INTRODUCTION

The question has often arisen as to how frequently diphtheria is a complicating factor in the early or later stages of scarlet fever among persons grouped together in hospital wards. Therefore, it was thought that it would be of interest to determine what percentage of scarlet fever cases, admitted to the Willard Parker Hospital, harbored diphtheria organisms and what proportion of these cases developed clinical symptoms of diphtheria. In the first series, cultures were made, unfortunately, only from the throats, but in the later series both nasal and throat cultures were taken.

HISTORICAL

Goodall[1] found that in 87 cases of scarlet fever examined on admission, 6 were found to have diphtheria-like bacilli in their throats. Pugh[2] found in 203 cases, from which cultures were taken on admission, 11 which had bacilli morphologically identical with the diphtheria bacillus, the organism from one case only being tested out, which proved virulent, thus showing that 5 per cent. had bacilli which could not be distinguished morphologically from the Klebs-Loeffler bacillus; B. hoffmanni was found in 41 cases or 20 per cent. of the cases. Of the 666 cases examined by Garrett and Washburn[3] at the Scarlet Fever Hospital in London, 8 or 1.2 per cent. were found to have bacilli morphologically like the diphtheria bacillus but no animal tests were made. Cumpston[4] found that in 1,019 cases of scarlet fever from which cultures were made on admission, that 75 or 7.36 per cent. showed diphtheria bacilli in nose and throat and 1.07 per cent. had B. hoffmanni. Of the 1,019 cases (the throat was examined in 1,017 cases), the nose was examined in only 165, 21 or 12.7 per cent. of which had diphtheria-like bacilli and 8 or 4.8 per cent. had B. hoffmanni. Of the 1,017 cases in which the throats were examined 59 or 5.8 per cent. had diphtheria-like bacilli and 7 or .68 per cent. had B. hoffmanni. Of the total 1,019 cases examined, 75 had diphtheria-like organisms on admission and of these 4 or 5.3 per cent. developed clinical diphtheria. With the exception of Pugh's one case, no mention is made of determining the virulence of these organisms.

The results of previous investigations on carriers by workers in this Laboratory have shown that a considerable percentage of diphtheria-like organisms are non-virulent.

TECHNIQUE

Cultures were made in the usual routine way from each patient on admission to the scarlet fever service and then sent to the Laboratory. After 24 hours incubation, agar plates and ascitic broth tubes were inoculated. All organisms morphologically like the diphtheria bacillus that were isolated, were tested out for virulence. Each test was made by in-

oculating subcutaneously two guinea pigs, each weighing about 250 gramms, with 1 c. c. of 48 hour ascitic broth culture; one pig receiving in addition to the broth culture ½ c. c. antitoxin which pig served as a control. If the strain was virulent, the uncontrolled pig died in less than four days and the culture was regarded as true diphtheria. If a culture was morphologically identical with the true diphtheria bacillus but did not prove virulent for guinea pigs when tested above, the strain was classed in the diphtheria-like group. Pseudo-diphtheria bacilli were differentiated morphologically only, though one or two strains in the first series were tested out which, of course, proved non-virulent.

Series I

In the throat cultures made on admission from 600 patients, bacilli morphologically identical with the diphtheria bacillus were isolated from 29 cases. From the animal tests made from these different strains, it was found that 12 out of the 29 were virulent for guinea pigs, thus showing that 2 per cent. of the cases on admission harbored virulent diphtheria bacilli in their throats and that 17, or 2.8 per cent., had organisms that could not be differentiated microscopically from the virulent type. There were eight cases which showed diphtheria-like organisms in the cultures but were not isolated, and 82, or 13 per cent., had B. hoffmanni or pseudo-diphtheria bacilli. These results correspond in part rather closely with the findings of Dr. Von Sholly[5], who examined the throats of 1,000 normal persons and found that 1.8 per cent. had virulent diphtheria bacilli in their throats, 3.8 per cent. non-virulent diphtheria-like bacilli and 26.6 per cent. had B. hoffmanni.

Of the 12 cases which had virulent diphtheria bacilli, 5 developed symptoms which since true diphtheria bacilli were isolated might be regarded as diphtheritic in character. These symptoms were enlarged tonsils covered with an exudate and in two cases accompanied with a profuse nasal discharge.

Series II

In the second series, cultures were made from the nose and throat, not only on admission, but after the pateients were admitted to the hospital if symptoms suspected of being diphtheritic in character developed. In some cases diphtheria-like bacilli were isolated from both nasal and throat cultures and when this occurred, the strain from the throat was first tested and if proved non-virulent, the nasal strain was injected into guinea pigs. Frequently, however, diphtheria bacilli were seen in both cultures, but it was possible to isolate from one source only, the diphtheria bacilli in the other culture being too few or the tube overgrown with other organisms.

Of the 336 patients from whom nasal and throat cultures were examined, 15, or approximately 4.5 per cent., were positive on admission, 8 of these cases having virulent bacilli and 7 non-virulent. None of these 15 patients, it is interesting to note, has developed clinical diphtheria, it being now two months since the last case was admitted with a positive culture.

Sources	Cultures Positive on Admission	Virulent	Non-virulent
Nose	7	7	3
Throat	2	1	4
Nose and throat	6	..	..
Total	15	8	7

It will be seen from the above table, that seven of the virulent strains were isolated from the nose and only one from the throat.

In the later cultures made from cases suspected to have developed diphtheria and who were negative on admission, there were 40 patients from which bacilli morphologically like the diphtheria bacillus were isolated and tested for virulence. Of these, 38 were true diphtheria bacilli and 2 diphtheria-like. Definite clinical symptoms developed in 23 of these cases, the other having only a suspicious nasal discharge, which were found to have true diphtheria bacilli, the symptoms appearing in several of the cases in one ward at the same time.

Later Cultures From Cases Negative on Admission

Source	Virulent	Non-virulent	Clinical Symptoms
Nose	14	2	11
Throat	24	..	12
Total	38	2	23

Summary and Conclusions

It is evident from the cultures examined in the first series that the percentage (2 per cent.) of scarlet fever patients admitted to the Willard Parker Hospital harboring virulent diphtheria bacilli in their throats is only slightly higher than that found in normal children, i. e., 1.8 per cent. In the second series of 336 cases in which both the nose and throat were examined, the percentage of cases having virulent bacilli is 2.3 per cent. Of these, however, 2 per cent. were from nasal cultures and only .29 per cent. were isolated from the throat. The low percentage of virulent organisms in these throats, may be due to the season in which the later series were examined, i. e., from June to October, inclusive, whereas the cases which gave the 2 per cent. of virulent throat cultures were examined from November to April. Although on admission each patient was immunized against diphtheria, 6.5 per cent. developed clinical symptoms and these occurred among cases which gave negative nose and throat cultures when admitted to the hospital.

The examination of more than nine hundred cases seems to warrant the following conclusions:

1. That the number of scarlet fever patients harboring virulent diphtheria bacilli on admission varies with the time of year that the patients are received at the hospital and does not differ greatly in the percentage of infected throats from that in healthy persons.

2. That in all routine examinations for determining the presence of the diphtheria bacillus, nasal cultures as well as throat cultures should be examined.

3. That, if possible, virulence tests should be made, as 50 per cent. or more of the cultures which are positive microscopically are non-virulent for guinea pigs.

4. That patients admitted to the hospital with virulent bacilli in their noses or throats do not, as a rule, develop diphtheria, if they receive an immunizing dose of antitoxin, but are of decided danger to the other patients.

TABLE I

1912–13	Number Examined	Diphtheria Bacillus	Diphtheria-like Bacillus (Non-virulent)	Hoffman's Bacillus	Diphtheria-like Bacillus (Not Isolated)
November	62	..	2	7	2
December	140	1	2	35	3
January	140	3	6	12	2
February	136	6	3	21	..
March	122	2	4	10	1
Total	600	12 (2%)	17 (2.8%)	85 (13%)	8

TABLE II

Ages	Diphtheria Bacillus	Diphtheria-like Bacillus (Non-virulent)	Diphtheria-like Bacillus (Not Isolated)	Hoffman's Bacillus
Under 1 year	..	..	..	..
1– 2 years	..	..	..	3
2– 3 years	4	..	1	7
3– 4 years	3	5	..	9
4– 5 years	1	1	2	11
5–10 years	3	4	2	34
10–15 years	..	4	2	10
15 and above	1	1	1	4
Age not given	..	2	..	4
Totals	12	17	8	82

1. Goodall, E. A.: Tr. Epidem. Soc., London, 1895, p. 68.
2. Pugh, W. T.: Metropol. Asyl. Board. Annual Report 1900, II. p. 201.
3. Garret and Washburn: Brit. Med. Jour., 1899, I, 893.
4. Cumpston, H.: Jour. Hyg., (Cambridge) 1907, VII, 593.
5. Von Sholly, A. I.: Jour. Infect. Dis., 1907, IV, 337.

THE VIABILITY OF THE B. PESTIS IN STOCK CULTURES

ROBERT J. WILSON

On November 18, 1899, cultures were made by W. H. Park, from the buboes of two cases of bubonic plague that had arrived at the Quarantine Station of the Port of New York on the Steamship "J. W. Taylor," from Santos, Brazil, on that date.

At this time studies of these organisms were being made at the laboratory and the keeping of the stock was in my charge. Our rule was to transfer the cultures at monthly intervals.

On June 8, 1903, a number of cultures that had been kept in the ice box unopened, from February 21, 1901, were subcultured and it was found that the organisms grew almost as readily as from recently planted cultures. At this time there were made a number of subcultures of three strains of *B. pestis* with the idea of transferring them at yearly intervals for a period of several years.

On November 14, 1913, there was made a transfer from a culture tube that had not been opened since June 8, 1903, a period of ten years and five months. After 48 hours there was an abundant growth of organisms which upon examination and subculture proved to be typical *B. pestis*. These organisms proved virulent to a guinea-pig after 48 hours, and the bacillus of pest was recovered in pure culture from the heart blood of the animal. This longevity of the organism is not confined to one strain, for a transplant grew from a culture of 9½ years old which was originally obtained from Kistasato's Laboratory. This culture was isolated by him from a case living in Hong Kong, China.

ON TUBERCULIN

James P. Atkinson and Charles B. Fitzpatrick

We have found that different preparations of tuberculin varied considerably in their physiological effects indicated by a change of arterial blood pressure when injected into the femoral vein of the dog. The first sample of tuberculin, tested on November 17, 1909, did not cause a fall in arterial pressure; which other samples, tested at a later period did produce. We found that if the unheated and unconcentrated cultures of the organism were filtered by means of a Berkfeld filter, and the clear filtrates were injected into animals, a marked drop in arterial pressure was produced, the same as occurred with the product concentrated at a rather low temperature. We made a series of experiments which showed that the depressor substance was thermostabile up to 100° C. even if kept at that temperature for one hour. If the temperature were raised and kept at 105° C. to 110° C. for one hour the depressor substance was destroyed or greatly diminished. Five different preparations of tuberculin were tested as to their action on the blood pressure. A marked variation in depressor values was found.

To test the action of heat on tuberculin a series of injections of the unheated and unconcentrated filtrate of a culture of b. tuberculosis passed through a Berkfeld filter was heated at different temperatures for one or two hour periods. Water was added afterwards to make the volume equal to the original volume.*

The following temperatures were used:

35° C. original incubator temperature.
50° C. — 2 hours.
60° C. — 1 hour.
70° C. — 1 hour.
80° C. — 1 hour.
100° C. — 1 hour.
105° C. — 1 hour.

The injections (5 ccm.) were begun with the preparations which had been heated to 105° C. and given in order to the original incubator filtrate. The 105° C. heated preparation gave only a slight indication of a reaction. The other preparations gave marked depressions.

Tuberculin heated to 105° C. — 110° C., as described, was tested on a tuberculous calf. The temperature of the calf was taken for three consecutive days previous to the injection of the tuberculin and, as the data presented shows, heating did not destroy the fever producing property of the sample.

Temperature of Tuberculous Calf:

May 18, 1 p. m. 102.6
May 18, 4 p. m. 102.4

* We are indebted to Mr. Banzhaf for the preparation of the heated samples.

May 18, 7 p. m.	101.8	
May 19, 9. a. m.	101.	
May 19, 12:50 p. m.	101.4	
May 19, 3 p. m.	101.	
May 20, 9 a. m.	101.4	
May 20, 12 m.	101.6	
May 22, 9 a. m.	101.2	—injected subcutaneously 1 c.c. crude tuberculin (heated at 110° C. for 1 hour).
May 22, 12 m.	101.	
May 22, 3 p. m.	101.6	
May 23, 8:20 a. m.	104.3	
May 23, 10:30 a. m.	104.6	
May 23, 12:30 p. m.	105.	
May 23, 2:30 p. m.	105.4	
May 23, 5 p. m.	104.6	
May 23, 6 p. m.	104.2	(Lying down)
May 23, 8 p. m.	104.2	
May 24, 9 a. m.	101.6	

Calmette's Opthalmo-Reaction.—The calf used in the above test was used later for the Calmette Opthalmo-reaction, with tuberculin heated at 110° C. for 1 hour. At 4 p. m. on January 18, 1912, 10 drops were placed on the eye; eighteen hours later an inflammation of the conjunctiva, with injection of the blood-vessels and the production of pus were observed.

The fact that "tuberculin" if not overheated is an arterial blood depressor suggests that the extract of the suprarenal gland might antagonize it. We have carried out experiments of this nature and find that according to the amount of tuberculin and suprarenal extract in the mixture one can produce a depression or rise at will. The depressor substance in tuberculin then can be measured by suprarenal extract of known strength.

The results indicate that tuberculin is a complex substance consisting of at least two poisons; one, a blood depressor destroyed by heat and antagonized by adrenalin; the other, a substance characterized by its fatal effect on tuberculous guinea pigs when injected subcutaneously and by its stability to heat (resisting a temperature to 105° C.).

ON SOME VASO-REACTING SUBSTANCES IN THE BLOOD SERUM OF RABBITS AFTER VACCINIA INOCULATION

James P. Atkinson and Charles B. Fitzpatrick

We have observed, in some previous work, that the blood serum of animals after certain infections has the property of causing a drop in the arterial blood pressure of the dog when injected directly into the circulatory system. Controls made with normal rabbit's serum from several rabbits did not show this phenomenon. After varying periods some of these infected animals were found to have developed a pressor substance in their serum. It was also observed that a serum which contained the depressor substance often lost it after aging in the ice-box.

We now present results of experiments made with the serum of rabbits after successful inoculation with vaccinia virus. The height of the production of the vesicles from which the virus was collected was reached on the fifth day. They then rapidly healed and the rabbits appeared to be in good health.

The dog was used to test the vaso-reacting properties of the sera. The pressures were taken with a mercury manometer from the carotid artery and the injections were given in the femoral vein.

The table gives the details of the experiments.

An examination shows that the sera drawn from rabbits 2, 8, 17 and 30 days respectively after inoculation, when tested not later than 6 days after bleeding, caused a drop in pressure.

The serum from the rabbit bled 2 days after inoculation aged for 21 days, also those bled 8, 17 and 30 days after inoculation aged 13 days, lost the depressor substances to the extent that either the serum caused a slight rise (followed by a slight fall), or a marked rise in pressure. The same phenomenon is observed in the sera drawn from rabbits 10 and 12 days after inoculation and aged 13 and 11 days respectively in the ice box. The serum drawn five days after inoculation and aged 18 days in the ice box caused a good rise in pressure.

Summary

1. Depressor and pressor substances arise after vaccinia infection in blood-serum of rabbits.

2. Aging tends to eliminate the depressor substance and a pressor substance then comes in evidence.

TABLE SHOWING WHEN THE DEPRESSOR AND PRESSOR SUBSTANCES APPEARED IN THE SERUM

Number of Experiments	Date of Experiment	Amount of Injection	Age of Serum	Drawn from Clot	Time after Inoculation with Vaccinia	Reaction
1	March 8, 1913	8 c.c.	24 hours	Within 12 hours of bleeding	48 hours	A very marked depression
2	" 28, "	6 "	21 days	" 12 " " "	48 "	Decided rise
2	" 28, "	5 "	18 "	" 48 " " "	5 days	A good rise in pressure
2	" 28, "	8 "	6 "	" 4 days " "	8 "	Marked depression
2	" 28, "	$2\frac{1}{2}$ "	13 "	" 4 " " "	10 "	Slight rise followed by a slight fall in pressure
2	" 28, "	4 "	11 "	" 4 " " "	12 "	Slight rise followed by a slight fall in pressure
2	" 28, "	5 "	6 "	" 4 " " "	17 "	Marked depression
2	" 28, "	5 "	6 "	" 4 " " "	30 "	Slight rise followed by a slight fall in pressure
3	April 4, "	8 "	13 "	" 4 " " "	8 "	Slight rise followed by a slight fall in pressure
3	" 4, "	8 "	13 "	" 4 " " "	17 "	Decided rise followed by an equal fall in pressure
3	" 4, "	8 "	13 "	" 4 " " "	30 "	A marked rise in pressure

THE FURTHER SEPARATION OF ANTITOXIN FROM ITS ASSOCIATED PROTEINS IN HORSE SERUM. NO. III

EDWIN J. BANZHAF

Without writing a lengthy historical account of what has been done before concerning the attempts at purifying the antitoxin, I will describe briefly a method of further purifying these substances. This method possesses the advantage of giving one finished product, whereas my former methods consisted of two fractions and two finished products, commonly designated as the A and B fraction or product. The two fractions necessitated two distinct methods for separation, precipitation and dialyzation. The new method shortens the process considerably, enabling one to finish the purifying, separation and dialyzing in about 10 days. A practical factor that one always has to contend with is the expense, and that is reduced. The finished product has, as a rule, a blue green color which is rather pleasing to the eyes. The deterioration of the finished product appears to be even less than the products from my former methods. In my first paper on the further separation of antitoxin from its proteins in horse serum.[1] I was the first to point out that pseudoglobulin could be converted into a euglobulin condition, *i. e.,* take the precipitation characteristics of euglobulin, without affecting the antitoxin, proving, thereby, that the antitoxin was not necessarily a pseudoglobulin, as it was formerly considered. The heating experiments of the pseudoglobulin containing antitoxin showed that when this solution was heated for 6 hours at 58° C., 30 per cent. of the pseudoglobulin was converted into a euglobulin condition. If a like solution was heated 12 hours, only 35 per cent. was converted, and when heated 24 hours, only 40 per cent. Instead of being converted in the same rate, as during the first 6 hours, the conversion curve, at longer heating, was greatly reduced. During the same time, *i. e.,* at longer heating, the curve of the destruction of antitoxin was greatly increased. It occurred to me that possibly the conversion curve of the pseudoglobulin into a euglobulin condition was lessened through the presence of the euglobulin in solution. As a matter of record I will state that the assumption appears true. If a solution of pseudoglobulin containing antitoxin is heated for 6 hours at 58° C., 30 per cent. of the pseudoglobulin will be converted into a euglobulin condition. If the euglobulin is salted out and the remaining pseudoglobulin dialyzed to about 0.8 per cent. salt content, and then heated again for 6 hours at 58° C., a conversion of 30 per cent. of the pseudoglobulin will again take place without much loss of antitoxin. Certainly this is interesting from a scientific standpoint. I hope to have more to write concerning this in the near future; at present it is far from a practicable method of purifying the antitoxin for therapeutic use. Casting about for a practicable method, it occurred to me that if the euglobulin was thrown out of solution as soon as it was formed more of the pseudoglobulin would be con-

[1]Banzhaf, Proc. of the Soc. for Exp. Biol. and Med., 1908—VI, 24.

verted. In order to test this, I added just sufficient amount of saturated solution of ammonium sulphate to the diluted plasma to precipitate all the euglobulin and then heated the mixture up to 60° C. The results showed that more of the pseudoglobulin was converted and that, if this heated mixture was filtered hot, the filtration was very rapid.

The limitations of these heating methods I have not up to the present time worked out sufficiently to report upon. It will be necessary at this time to call attention to the fact that when a mixture of plasma and ammonium sulphate is heated the precipitation limits of the proteins are changed. For instance, a serum of plasma diluted in such a manner as to contain about 6 per cent. proteins will require 32 per cent. saturated solution ammonium sulphate to precipitate all the euglobulin. The addition of the ammonium sulphate solution will bring the protein content of the mixture down to about 4 per cent. Where the mixture of plasma and ammonium sulphate is to be heated up to 60° C., the following holds true; with the plasma diluted to contain about 6 per cent. protein, the addition of only 30 per cent. saturated solution will be required to bring about like results.

The following method for purifying and concentrating is very practicable for therapeutic use:

The citrated plasma is diluted one-half its volume with water and saturated solution ammonium sulphate is added up to 30 per cent. saturated solution, *i. e.,* 7 cubic centimeters diluted plasma and 3 cubic centimeters saturated solution ammonium sulphate. This dilutes the plasma so that the solution now contains a little less than one-half the amount of protein in the original plasma. The total protein in the plasma of immunized horses will vary from 8 to 10 per cent.[2] Plasma diluted in the above manner will contain from 4 to 5 per cent. protein. With this amount of protein, the 30 per cent. saturated solution of ammonium sulphate will precipitate, when this mixture is heated, all the fibrin and all the euglobulin. In cases where the original plasma contains 10 per cent. protein, a small amount of pseudoglobulin and antitoxin will also be precipitated. The amount of antitoxin pulled down is so small that it can be disregarded for the practicable method of purifying. It is also true that in cases where the orginal plasma contains the lower percentages of protein, 8 per cent. or below a small amount of the euglobulin is not precipitated, but this, too, can be disregarded. As a rule, however, where large lots of plasma are used from different bleedings and different horses the protein content will average about 9 per cent. and the antitoxin will not be precipitated nor will euglobulin remain in solution.

The diluted plasma containing the 30 per cent. saturated solution ammonium sulphate is heated up to 60° C. in agate pots of 30 liters capacity. The hot water bath in which these pots are placed may be kept at 66° C. The length of time to heat the plasma ammonium sulphate solution, *i. e.,* to bring it up to 60° C., should take about two hours for the best results. It is essential to keep the pots covered during the

[2]Banzhaf and Gibson, Coll. Studies, Research Lab. Dept. of Health, 1908—9, IV, 202.

heating to prevent loss of water through evaporation. If loss of water was allowed to take place, the concentration of ammonium sulphate would be increased and antitoxin would be precipitated. It is also essential to stir the mixture frequently to insure uniform heating. As soon as the mixture reaches 60° C., the pots are to be removed from the bath and the contents filtered immediately. The glass funnels and glass precipitating jars, if these are used in filtering, should be warmed to prevent cracking just prior to receiving the hot plasma and ammonium sulphate mixture. The filtration will be very rapid. (80 liters distributed on 10 funnels filtered through in less than 2 hours.) The precipitate containing the fibrin and euglobulin, although filtration has practically ceased, will retain considerable of the fluid which contains pseudoglobulin and antitoxin. There are several methods of reclaiming this fluid. A very practicable method is to press the precipitate in the same manner as in the final precipitate of pseudoglobulin and antitoxin, but, in this case, catching the fluid pressed out. This method is very satisfactory and very rapid, but does require experience to obtain good results in reclaiming all of the clear fluid. This clear fluid is of course added to the main filtrate. Another method of reclaiming the fluid from the precipitate in instances where the pressing facilities and recovery of the fluid are not of the best is to add water containing 33 per cent. saturated solution ammonium sulphate to the funnels containing the precipitates, thereby washing them. A more thorough manner would be to place the precipitates in the water containing the ammonium sulphate, mixing the whole well and refiltering. The resulting filtrate can then be added to the main filtrate and the precipitate of fibrin and euglobulin thrown away. It is to be remembered that this washing must be carried out with water containing 33 per cent. saturated solution ammonium sulphate and not 30 per cent. The reason for this is that the solubility of the euglobulin is increased, due to the fact that there will be less protein in solution. The saturated solution of ammonium sulphate of the combined filtrate may be regarded as 30 per cent. Sufficient saturated solution of ammonium sulphate is added to raise the regarded content of the combined filtrates from 30 per cent. up to 50 per cent. The resulting precipitate will contain all the pseudoglobulin and antitoxin. After filtering, this precipitate is pressed to remove excess of fluid and then dialyzed until free from ammonium sulphate.

THE ACID AGGLUTINATION OF SENSITIZED BACTERIA

Charles Krumwiede, Jr., and Josephine S. Pratt

In the Deutsche Medizinische Wochenschrift, No. 21, May, 1911, Michaelis reported a series of experiments on the acid agglutination of bacteria. He concludes: "Many species of bacteria are agglutinated by acids in such a fashion that a definite degree of acidity, best measured by the concentration of hydrogen ions, corresponds to the optimum agglutination. This optimum is characteristic for each variety of bacteria and can be used as an aid in identification."

The following is an example of the method employed. A series of solutions with increasing amounts of acid are used. From formulae given by Michaelis the hydrogen ionsization in these solutions can be determined. We have worked with the following mixtures:

Solutions	I	II	III	IV	V	VI
Normal sodium hydrate	5. c.cm.	5 c.cm.	5 c.cm.	5 c.cm.	5 c.cm.	5 c.cm.
Normal acetic acid	7.5 "	10 "	15 "	25 "	45 "	85 "
Water	87.5 "	85 "	80 "	70 "	50 "	10 "

To a series of six tubes is added 1 c. c. of these mixtures and 3 c. c. of distilled water suspension of the bacterium to be tested. The tubes are then incubated until evidence of agglutination can be noted. The further progress of the agglutination may be observed at room temperature.

The following table shows an average reaction of two closely allied bacteria.

	I	II	III	IV	V	VI
Typhoid	−	±	+	+	±	−
Para typhoid	−	−	−	−	+	+

Before the report of Michaelis' work, much had been done to explain agglutination as a physical phenomenon. The demonstration by Bordet that agglutinins combining with bacteria cause no agglutination unless a salt be present, was the starting point of this work.

The similiarity of this reaction to the action of salts on colloidal solutions led to much comparative work on the action of physical agents, such as acids, salts and the electric current on colloids and bacteria. Likewise, the changed reaction to such agents of bacteria, after absorbing agglutinins, was investigated by Neisser and Friedman and Bechhold. This work was further elaborated by Buxton and his associates.* The chief point of interest to us was the increased sensitiveness of sensitized bacteria to the action of the various agents, especially acids.

* For theoretical discussion, résumé of earlier work and bibliography, see Beinasch, Zeitsch. Für Immunitätsforschung. Orig. Bd. 12, 1912, p. 268, and also articles by Buxton and associates, Studies, Department of Pathology, Cornell University, Vol. VI. 1906.

Considering these results, it appeared to us that if bacteria were sensitized their point of acid agglutination should differ from that of normal bacteria. Further, we wished to determine whether this change could be brought about by the application of amounts of serum smaller than were necessary to cause ordinary serum agglutination. In other words, whether the changed reaction to acids would reveal traces of agglutinins, the presence of which could not be determined by the ordinary methods.

We have studied this reaction, using old and freshly isolated strains of typhoid bacilli. A high titre typhoid serum obtained from a horse was employed and as controls, the sera of normal horses.

As our work was done with only one variety of organism, we cannot say whether the method is of practical value for differentiation. The results, however, seem of sufficient theoretical value to warrant us in making this report.

The method used was as follows:

To complete the sensitization the mixtures were left one hour or longer in the incubator. The different dilutions of the serum with bacteria were added to series of tubes containing the acid solutions already described, and in every series was added, as control, a tube with distilled water instead of the serum dilution.

Generally, the tubes for the serum agglutination were left for one hour in the incubator and readings were made after allowing the tubes to stand for one hour and over night at room temperature. For acid agglutination the bacteria were sensitized one hour, the acid mixture added, and the tubes incubated one-half hour longer. The reaction was read, after one hour and after over night, as with the serum alone. The following table illustrates the difference between sensitized and untreated bacteria:

	I	II	III	IV	V	VI
Typhoid	−	±	+	+	±	−
Sensitized typhoid	+	+	+	+	+	+

The most marked difference was in Solution I. In this solution agglutination of our typhoid culture was never observed. Some, however, showed a slight reaction in Solution VI. In our later work we used only Solutions I and II. The following table gives our results. Where agglutination took place in Solution I the results are called positive.

Typhoid (Mt. Sinai)

Typhoid Serum	Agglutination	Acid-agglutination
1:10,000	±*	+
1:20,000	|	±
1:25,000	−	±
1:50,000	−	|

* Complete agglutination +
Marked " ±
Slight " |

The dilutions recorded are the highest which showed the degree of agglutination indicated by the symbols. After standing overnight the following were the results:

Typhoid Serum	Agglutination	Acid-agglutination
1:10,000	+	+
1:30,000	±	+
1:40,000	|	+
1:100,000	−	|

Another series of tests was prepared at the same time as the preceding, using the same bacterial suspension and serum dilutions. These were sensitized for one hour in the incubator and then left over night at room temperature before adding to the acid solutions. The results were as follows:

Acid agglutination—

1:30,000±; 1:40,000 |.

After standing over night at room temperature the following readings were made:

Acid agglutination—

1:40,000±; 1:50,000 |.

Repeating this test gave similar results.

It is evident that sensitization for one hour at 37.5 C. is sufficient, and that longer action does not cause acid agglutination of bacilli sensitized with higher dilutions. The following tests, using the strain Mt. Sinai, were made to determine how short a time sufficed to sensitize the bacteria:

	Immediately after incubation		Over night at room temperature	
Serum agglutination	1:10,000	|	1:10,000 1:17,500	± |
Acid agglutination (sensitized one hour)	1:15,000 1:30,000 1:40,000	+ ± |	1:30,000 1:50,000	+ |
Acid agglutination (momentary sensitization)	1:20,000 1:30,000	+ |	1:30,000 1:40,000	+ |

Mixing the acid solution and the serum dilution and immediately adding the bacterial suspension or incubating the mixture of acid solution and serum dilution before the bacteria are added makes the reaction much less sensitive than when bacteria and serum are mixed first and the acid added later. It is probable that the acid has an unfavorable action on the serum. Rapid sensitization for the period of only a minute is nearly as efficient as when continued longer, that is, most of the antigen and anti-

body are bound in a short time. We continued therefore to sensitize for one hour.

Strain	After 1½ hours		The morning after	
	Serum-agglutination	Acid-agglutination	Serum-agglutination	Acid-agglutination
Typhoid 100	1 : 10,000 —	1 : 10,000 ± 1 : 17,500 —	1 : 10,000 —	1 : 12,500 + 1 : 15,000 ± 1 : 20,000 |
" 103	1 : 10,000 ±	1 : 10,000 + 1 : 17,500 ±	1 : 15,000 ±	1 : 20,000 + 1 : 30,000 ±
" 104	1 : 12,500 ±	1 : 15,000 ±	1 : 15,000 ±	1 : 20,000 ±
" 105	1 : 10,000 —	1 : 12,500 ±[1]	1 : 10,000 —	1 : 12,500 +[1]
" 106	1 : 6,000 ± 1 : 15,000 |	1 : 6,000 ± 1 : 15,000 ± 1 : 30,000 ±	1 : 6,000 ± 1 : 15,000 |	1 : 6,000 ± 1 : 40,000 ± 1 : 50,000 |
Typhoid 112 (freshly isolated from a carrier, 106 from same case)	1 : 10,000 —	1 : 10,000 ±	1 : 10,000 —	1 : 10,000 ± 1 : 20,000 |
Typhoid 113 (freshly isolated)	1 : 15,000 ±	1 : 10,000 ±	1 : 15,000 ±	1 : 17,500 ±
3 months later	1 : 15,000 |	1 : 15,000 |	1 : 20,000 |	1 : 20,000 ± 1 : 30,000 ± 1 : 40,000 |
	After another hour's incubation, as the control showed only slight agglutination			
	1 : 17,500 |	1 : 17,500 ± 1 : 30,000 ±		
The same, repeated	After 2½ hours in incubator	After 1½ hours in incubator		
	1 : 20,000 ± 1 : 25,000 —	1 : 20,000 ± 1 : 30,000 ±	1 : 20,000 ± 1 : 25,000 |	1 : 20,000 ± 1 : 50,000 ±

[1]Not tested in higher dilutions.

Two of our strains, Nos. 104 and 113, were very resistant to acid agglutination, and did not lose this property even after sensitization. We believe, therefore, that the acid serum agglutination occurs with highest dilutions where the strains show a rapid acid agglutination.

The contrast between the degree of the serum and acid agglutination of sensitized bacteria was especially marked in those cultures which were promptly agglutinated by the acid. As already stated, rapid momentary sensitization is nearly as effective as sensitization for one hour. With easily agglutinated strains the acid agglutination of the sensitized bacteria takes place promptly, that is, after about one-half hour incubation. This occurs even in high dilutions. On the other hand, even if the strains are easily agglutinated by serum alone, only low dilutions show agglutination after such short incubation.

For controls we used various normal horse sera and tested their influence on our typhoid strains. Normal horse serum in a dilution of

1:100 inhibited the acid agglutination of one of our strains; even in dilutions of 1:1000 the sera showed this inhibition to a slight degree. The inhibition with this strain was evident with one serum even in a dilution of 1:20,000, and this was shown although the acid was added immediately to the serum-bacteria mixture. The acid agglutination of other strains was somewhat inhibited by this serum. All other strains were promptly agglutinated in Solution I, with low dilutions of normal horse serum, that is, they changed the acid agglutination point like an immune serum. The above-mentioned serum was an exception. This changed reaction did not take place in dilution over 1:1000. None of the normal sera agglutinated typhoid directly in a dilution of 1:50.

Heating normal serum for one hour at 50° C. did not change the influence of the serum upon the acid agglutination. The serum was diluted 1:10 and after adding B. Coli incubated one hour and passed through a Berkefeld filter. Its influence upon B. typhosus was only slightly weakened.

We studied the influence of typhoid serum on the acid agglutination of B. paratyphosus, using only two strains, Schottmüller Muller and Seeman. The results were the same as with normal serum. Both showed an influence in dilutions up to 1:5000, that is, acid agglutination occurred in lower concentrations of the acid than with normal bacilli.

Conclusions

Sensitized typhoid bacilli are agglutinated in Solution I, which does not agglutinate the normal bacilli. In general this change takes place with higher dilutions of the serum than those which cause ordinary agglutination. In some cases normal serum causes the same change in dilutions up to 1:1000. In others it inhibits the acid agglutination where it normally occurs even in high dilutions. Only the sera from immunized animals agglutinating in high dilutions therefore could be used to show the specific reaction. Whether the changed acid agglutination as a reaction has a practical value must still be proven, also it must be determined if there is an increase in the limits between specific and group reactions. It is possible that bacteria which show similar acid or serum agglutinations might be differentiated by acid-serum agglutination. It would be of interest to determine what influence the sensitization of antigens, other than bacterial would have on their acid agglutination.

The action of normal serum must be carefully controlled. The existence of a non-specific reaction of normal serum is of interest from the standpoint of the physical theory of agglutination. This action should be considered in the theoritical conclusions regarding the influence of immune serum on the reaction of bacteria to physical agents.

OBSERVATIONS ON AN EPIDEMIC OF CONJUNCTIVITIS IN AN INSTITUTION

By Percy R. Crane

At an institution for feeble-minded children within 75 miles of New York City, in the early months of 1912, occurred an epidemic of inflammatory conditions of the eyelids which, until isolation was enforced, was continually spreading. The infected individuals were the basis of considerable study, which formed a part of the work on trachoma carried on by a group of workers in the Research Laboratory of New York City Health Department.

Seventy-nine cases were presented, and in many cases the clinical appearances were strikingly similar. In these the principal points were considerable swelling of the lids, mucopurulent discharge, and a rough or velvety appearance and beefy-red color of the palpebral conjunctiva. None originally had the large follicles that are so common in our school children. Several were complicated by pathological conditions of the cornea. Cultures and spreads of these cases were made by the method described on page 222.

The smears were given a very thorough examination, especially for bacteria and for the so-called trachoma inclusions (p. 230).

The fact that very few of these inclusions may be present in a smear necessitates often long search of the entire slide. This is greatly facilitated by the use of the mechanical stage and by a preliminary examination with the low-power lens which usually brings out the inclusions very distinctly. By using the high-power lens only to corroborate the low-power findings much time is saved. Under the high power the individual elements of the groups may show clearly, particularly about the periphery. The granules show absolutely no resemblance to eosinophilic protoplasm granules or other cell degenerations. The granules often have the appearance of very small micrococci or bacilli but are usually so closely packed together that it may be impossible to distinguish the individual elements. In the earliest stages the group may consist of but few individual elements (the "initial bodies" of Lindner), staining a deep blue with Giemsa. The clear halo surrounding the granules described by some observers I have found by no means constant.

In the later stages the groups are larger, the individual elements are increased in number, and the color is violet or even magenta instead of blue. In the terminal stage the granules may be diffusely scattered through the cells and almost fill the entire cytoplasm. One cell may and often does contain several groups of granules. They may lie anywhere within the cytoplasm, but I have found them perhaps more frequently directly adjacent to the nucleus. I have seen none that were intranuclear.

In some cases the granules have assumed more of the coccus or bacillus type, *i. e.*, are slightly larger or longer than those found in the usual groups. Dr. Williams has shown the similarity in appearance between

stained preparations from old cultures of certain haemoglobinophilic bacteria and inclusions (p. 230). Reasoning from morphologic appearance alone, it would seem that the inclusions originated directly from bacteria. This would tend to disprove the theories that the bodies are chlamydozoa or are cell reaction products.

The significance of these bodies in inflammatory affections of the eyelids has been widely discussed with much variation in opinion. The large majority of the cases which I desire to report, while presenting similar clinical appearances when first seen, ranged in duration from one or more days to several years. Thirty-one cases had lasted from 3 to 6 weeks and showed a thickened mucous membrane (papillary condition). These were termed acute. In all of these cases, except two, trachoma inclusions were found, which in some were very numerous. Of the longer standing cases (1½ months to several years) 9 showed the bodies and 7 did not. This confirms the fact noted by others that the inclusions are increasingly difficult to find in direct proportion to the age of the case. Among the cases reported upon it was noted that the bodies disappeared in cases under active treatment, such as the application of blue-stone, bichloride rubbing, etc. When first seen, some of the older cases had never received treatment, and most of the others had been without it for several months.

One of the most significant observations in this group of cases is that among the 32 cases diagnosed as acute catarrhal, and, moreover, among the 25 of these that recovered apparently completely in about 2 months 7 had typical trachoma inclusions.

The number of haemoglobinophilic bacilli found (in 26 cases) is also significant (see table showing coincidence of haemoglobinophilic bacilli and inclusions and termination of cases). As most of these eyes had been neglected, there were many staphylococci present, which, according to the results obtained in the recent cultural work of the Research Laboratory on trachoma (p. 229), may have interfered in many cases with the isolation of the haemoglobinophilic organisms.

Most of the cases with trachomatous complications or sequelae showed the inclusions; *i. e.*, cicatricial tissue, 2 cases, both positive; etropon, 3 cases, 2 positive, 1 negative; pannus, 1 case, positive; keratitis, 3 cases, 1 positive, 1 negative, 1 doubtful; corneal opacities, 2 cases, both positive; corneal ulcers, 1 case, negative; ptosis, 4 cases, all positive. Two cases with blepharitis were positive.

In the whole group were 5 cases of phlyctenular conjunctivitis. In none of these were inclusions found. One of these cases had a hypertrophic condition and was complicated by corneal opacities; all were rapidly cured, except the last.

Most of the cases that showed inclusions presented a rough, granular, velvety or papillary condition of the lids.

The significance of the microscopic findings in this group of cases seems evident. Unfortunately some of the slides did not stain well, and it may be possible that some of them that were denominated negative should have been positive. Also the previous duration of the cases as given in some instances may be inaccurate. Making due allowances, however, it cannot be denied that the search for the inclusions is a valuable procedure

for determining prognosis in inflammatory conditions of the eyelids accompanied by a thickened (hyperplastic?) conjunctiva.

Bacteria (staphylococci? and haemoglobinophilic bacilli?) were commonly found in the slides at the commencement of these observations and gradually grew less or disappeared as the cases succumbed to treatment. As previously mentioned, morphologically speaking, transition forms between inclusions and small bacilli were noted in several cases.

The infectivity of the cases presented was exceedingly high. Before isolation new ones were the usual thing every day. Nearly all had profuse mucopurulent discharge.

Unquestionably cases of the type mentioned in this report—actively secreting cases—are highly infectious. Though the children slept in separate beds and used separate towels and pillow cases, ample means for the transfer of the secretion by the hands, on the school books, toys, etc., were present. The fact that these children were feeble minded made the carrying out of directions for personal hygiene difficult.

In the treatment of these cases cleanliness was emphasized as by far the most important point. The use of boric acid (sat. sol.) was advised, every hour if necessary, to keep the eyes free from secretion. Argyrol, 20 per cent sol., or silver nitrate, 2 per cent sol., were the remedies employed in the acute stage (that is, during marked congestion and discharge). The children with unilateral disease were carefully warned of the danger of infecting the healthy eye, and were instructed accordingly. Yellow oxide ointment was used in cases of blepharitis. Upon the subsidence of the acute symptoms, the caustics, blue-stone and alum, or the friction treatment (briskly rubbing the lids with swabs saturated with bichloride 1-500), were the measures used. Rubber gloves were worn during the treatments, sterilized by dipping the hands in bichloride solution before each case was treated; and individual copper or alum pencils were used for each eye, previously sterilized in 95 per cent. alcohol. Care was taken not to allow the tip of the pipette containing argyrol or silver nitrate to touch the lids or secretions. Following out the plan used in the research work a change of treatment was made at intervals, and it was found to give better results than one remedy used continually, and this was done in these cases. As recovery ensued, examination of the eyes for refractive errors was recommended.

I have spoken of the treatment in only a general way, individual variation being made to suit the needs of each case. Hot applications and atropine were used in those complicated by active corneal ulcer, keratitis and secondary iritis. Ingrowing eye lashes were removed, in the cases of cicatricial entropion, and subsequent operation advised. Surgical measures were also recommended in a case of entropion in which persistent lachrimation was the result of eversion of the puncta.

The result of this management of the cases here reported was soon shown by a marked improvement. In almost all, the acute symptoms subsided, the discharge diminished or disappeared, and the lids became paler. When strict isolation was enforced, no new case broke out.

Theoretically segregation of all cases with secretion would be the ideal prophylactic, but of course this is impracticable under many conditions.

Clinical Diagnosis	Number of Cases	Microscopic Findings	Cultural Findings	Termination							
		Trachoma Inclusions	Haemoglobinophilic Bacilli	Improved		Unimproved	Recovered				
				9 mos.	3 mos.	9 mos.	9 mos.	4 mos.	3 mos.	2 mos.	2 wks.
Chronic trachoma.....	16	9	4 (1 in smear)	8	..	7	1				
Acute papillary conjunctivitis.....	31	29	12 (2 in smears)	17	2	1	6	4	1		
Acute catarrhal conjunctivitis.....	32	7	10	..	..	..	1	3	1	25	2
	79	45	26								

GENERAL GONOCOCCUS INFECTION IN A MALE CHILD WITHOUT EVIDENCE OF URETHRITIS

MATTHIAS NICOLL, JR., and MINNIE AUGUSTA WILSON

The patient, a boy aged two and one-half years, was admitted to the Scarlet Fever Hospital of the Department of Health on the service of Dr. Alfred Hess on June 14. He gave an indefinite history of scarlet fever three weeks previously.

Physical examination.—No eruption on body. Desquamation on palms and soles. General anasarca. Face and eyelids very puffy. Heart: soft, systolic whiff over the aortic area. Second sound accenutated. Area of cardiac dullness increased three centimeters to the left. Lungs: Slightly high-pitched breathing over the left apex in front. Temperature: 100° F., pulse 170, respiration 22. Urine: Sp. Gr. 1022, acid, marked trace of albumin, many granular casts, much free blood. The patient was very apathetic.

Subsequent condition.—During the next few days the condition of the kidneys showed marked improvement under diuretic and diophoretic treatment. The pulse, however, continued to be very rapid.

June 27, the left elbow and knee became swollen and very tender, evidently containing fluid. This was preceded for several days by a marked rise of temperature, 102° to 103° F. The condition was regarded as due to a scarlatinal arthritis. During the next two weeks the affected joints continued to be in practically the same condition. The pulse was rapid, the afternoon temperature 101° to 102°. The patient was extremely emaciated.

July 17, there was an exudation on both tonsils and posterior pharynx. Cultures showed diphtheria bacilli. Ten thousand units of antitoxin were given. This condition cleared rapidly. There was a swelling, the size of a hen's egg, over the right sterno-clavicular articulation, which developed within a few days into an abscess from which pus, aspirated under conditions to prevent contamination, was sent to the laboratory for examination. Gonococci were found to be present. The abscess was then opened and drained. The temperature and pulse, which had risen during the formation of the abscess, fell somewhat after the operation. A blood culture taken on this day to determine if general sepsis was present proved negative.

July 24, the left shoulder was swollen and painful. It was incised and drained five days later, a quantity of thick pus evacuated and sent to the laboratory for examination, and gonococci found.

August 4, as the pressure of gonococci was definitely determined in the abscesses, vaccine treatment was begun, 100,000,000 of a polyvalent preparation being given.

August 9, a blood culture to determine the presence of gonococci proved negative. At the time of beginning specific treatment the open wound in the sterno-clavicular regions was practically healed. The opening about the left shoulder joint showed flabby granulations and much

discharge. The left knee joint was greatly swollen and contained fluid. It was exceedingly tender to the touch or on attempting passive movement. The leg was held stiffly at right angles. The left elbow joint showed practically an identical condition. The patient was greatly emaciated. A week after beginning treatment the local and general condition began to improve rapidly, the swelling and tenderness growing less from day to day and the patient began to eat and gain flesh. The course of vaccines is indicated on the temperature chart.

At the time of his discharge from the hospital on August 24 he was fairly well nourished and seemed well. The involved joints were moderately thickened. There was little or no pain on active or passive movement. There was, however, in the elbow joint marked crepitation

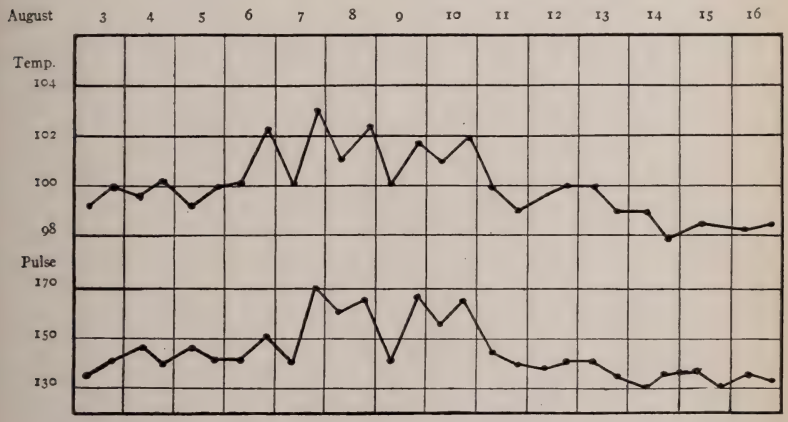


Chart 1.—Temperature and pulse curves.
Vaccine administered August 4, 7, 9 and 14 in doses of 100 million, 100 million, 150 million and 200 million strength, respectively.
Blood culture made August 9.

on passive movement, probably an evidence of some permanent damage. The wound in the shoulder had been completely healed for some 10 days. There was no apparent abnormality to be made out in the heart although the pulse rate continued above normal.

During the patient's stay in the hospital a number of small abscesses developed about the face and extremities. Cultures from them showed only staphylococci, and they healed readily after incisions. A circumscribed necrosis opposite two teeth in the lower jaw yielded rather slowly to local applications. Smears from the necrotic area showed mixed bacteria with many fusiform bacilli and spirochaetes.

There had never been the slightest evidence of urethritis as shown by discharge or difficulty in urination during the patient's stay in the hospital nor had the parents noted any such symptoms before his admission. With the object, however, of determining the point of entry of the invading organism, a number of urethral cultures were made together with one from the tonsils. The latter proved negative. The results of the former together with those from the affected joints are as follows:

Urethra.—1st culture—unsatisfactory, negative.

2d culture—gave a gram negative bacillus.

3d culture—on ascitic plates showed typical gonococcus colonies, fishings from which gave gram negative cocci, morphologically identical with gonococci.

4th culture—negative.

5th culture—smear from swab showed intracellular gram negative diplococci, cultures from swab on ascitic agar plates gave gonococcus colonies after 24 hours, fishings from which showed a pure culture of morphologically typical gonococci.

Sterno-clavicular Joint.—Cultures showed a symbiotic growth of gram negative diplococci and gram negative bacilli; the former could not be isolated in pure cultures.

Shoulder.—Smears from the pus showed intracellular gram negative diplococci. Blood-streaked agar plates after 48 hours showed typical gonococcus colonies, fishings from which gave morphologically typical gonococci in pure culture.

Complement fixation.—Pure cultures of the gram negative cocci from the shoulder and urethra autolyzed at 56° in distilled water for 24 hours used as an antigen and tested against gonococcus immune rabbit's and horse's blood gave in each instance a high degree of fixation.

Conclusions

1. Although two blood cultures failed to show the gonococcus, it is evident that the blood stream carried this organism to the widely distant points of localized infection.

2. The gram negative bacillus found in symbiosis with the gram negative coccus in the sterno-clavicular joint was in all probability identical with that found in the urethra and carried from it to the joint together with the gonococcus.

3. The fact that two of the joints did not suppurate is no reason for regarding their condition as due to the invasion of other organisms than the gonococcus, especially in view of the promptly beneficial action on them produced by specific treatment.

4. The presence of virulent gonococci in the male urethra does not necessarily cause recognizable local symptoms.

Since the writing of this article attention has been called to one by Dr. L. Emmett[1] in which he reports the occurrence of an epidemic of gonorrhoeal arthritis in young children among whom were 19 males. In only one of them was there evidence of gonorrhoeal infection elsewhere—a case of ophthalmia. Repeated cultures made with the object of determining the point of infection, from the urethra, nose, eyes and mouth all proved negative.

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[1]N. Y. Med. Jour., Vol. 81, 1905, March 18 and 25.

A CASE OF MASTOIDITIS FOLLOWED BY GENERAL SEPSIS, WITH SYMPTOMS OF TETANUS*

By Matthias Nicoll, Jr., and Frank S. Fielder

The following case, illustrating extreme difficulty in diagnosis even with every facility for careful study, seems worthy of report.

The patient, Olympia V., age four years, was sent to Bellevue Hospital on January 6, 1911, as a case of tetanus and probably due to vaccination two weeks previously. As the vaccine used was that sent out by the Department Laboratory, the case was turned over to us for advice and treatment.

Past History—Patient has always been well.

Present Illness—Vaccinated two weeks ago. Two days ago she became drowsy, unable to open mouth or take solid food. Yesterday began to have convulsions and has them at irregular but frequent intervals.

Physical Examination—General appearance well developed and well-nourished child. Unconscious, cyanotic, lies flat on back, occasionally moaning.

Eyes—Pupils equal, react to light. Lids kept half shut. Tendency to keep eyes turned to the left. No paralyses.

Head well formed, no sign of traumatism. Jaws set tight. On attempting to open them a clonus is set up.

Respiration markedly irregular, occasionally sighing. The rate not increased, no dyspnea.

Heart—Sounds normal, not enlarged, force good.

Extremities—Well formed, reflexes over-acting. Ankle and patellar clonus easily obtained. Hands, fingers and wrists in position of tetany. No Babinsky, no Kernig, moderate rigidity.

Muscles—Fibrillary contraction of platysmae.

Skin—On right upper arm there is a hyperaemic area slightly indurated, about the size of a silver dollar, in the center of which there is a crust about the size of a 5 cent piece. Scattered about the crust are a few small vesicles. The lesion has the appearance of a perfectly normal vaccination in the drying stage.

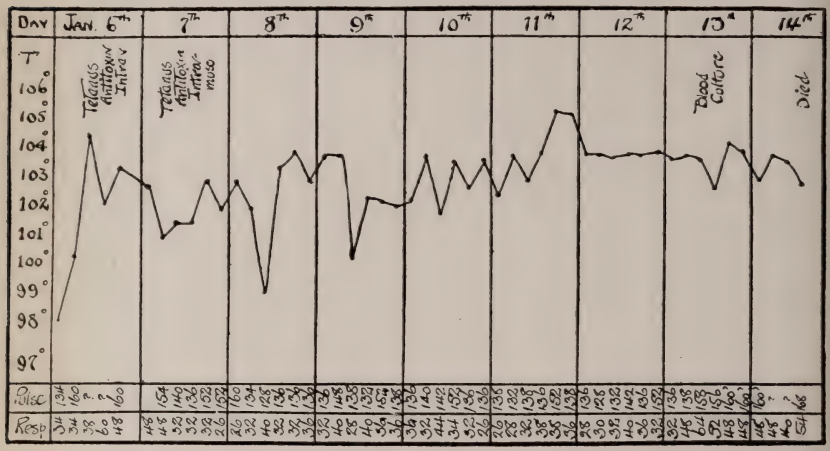
Pulse—Moderate rate, high tension and regular.

During the forenoon of January 6th, 17,000 units of Tetanus antitoxin were given intravenously. The material from the vaccination was removed to the laboratory for study. The stock of vaccine with which the child had been vaccinated was resubmitted to a test for tetanus similar to that employed on each speciman of virus before its issue. The result was negative by culture and animal inoculation in both instances.

During the day of January 6th, and the following night and day, the child continued in the condition in which she was received into the hospital—unconscious, temperature 101° to 103° F., heart action rapid and feeble, trsimus marked, slight left strabismus in both eyes. Hand

*Abstracted from an article published in the Archives of Pediatrics, August, 1912.

and arms in condition of tetany, more marked on the right side. No rigidity of neck, back muscles or lower extremities. Occasional convulsive movements of the face and upper extremities, but no general convulsions. The child was being fed through the nose.



Temperature Chart of Case Reported.

Lumbar puncture was performed and about 30 c. c. of clear fluid under moderate pressure were obtained. Examination showed fifteen cells to a cubic millimeter, globulin test negative. Fehling's solution reduced. No meningococci or tubercle bacilli. Blood pressure: systolic, 60; diastolic, 38.

January 8th, the rigidity has diminished. Less spasm of jaws, no clonus obtained on attempting to open them. No ankle or patellar clonus obtained to-day. No fibrillation of platysmae. The child remained unconscious. Babinsky sign obtained on the left side. Has vomited several times to-day. The bowels are loose and move frequently. Several convulsions of a mild grade.

During the night, the child had occasional muscular twitchings of face and eyes principally on the right side, and of the right upper extremities. The jaws are still stiff. In the afternoon, an examination of the ears showed some redness and slight bulging of left tympanic membrane. Right drum apparently normal. Paracentesis of left drum produced free bleeding but no pus. Within twenty-four hours the irrigation fluid returned clear.

January 11th, eye examinations by the visiting ophthalmologist showed no abnormality.

During the next three days the patient grew weaker, with diarrhea and occasional vomiting; was still unconscious, but could be aroused to a certain extent. There was alternate pallor and flushing of the face. The trismus persisted but was less marked. Rigidity of the arms and hands less; none of the neck, back or lower extremities.

January 13th. In the morning the left foot and left hands were observed to be markedly cyanotic. A blood culture taken showed hemolyzing streptococci in abundance.

Death occurred in the afternoon, eleven or twelve days after the onset of first symptoms, eight days after admission to the hospital.

The urine had been passed in sufficient quantities and the examination of it showed nothing abnormal. The autopsy findings reported by Dr. Norris, director of the pathological department of Bellevue Hospital, revealed the following conditions:

Double suppurative otitis media and mastoiditis; edema (inflammatory) of scalp; petechiae of the pleura; commencing hemorrhagic lobular pneumonia; persistent thymus; acute parenchymatous hepatitis and nephritis; moderate pial edema; streptococcic septicaemia.

The following lesions are of sufficient importance to give in detail.

There is moderate pial oedema. The vessels at the base are normal. The anterior cisterna contains clear fluid. The lateral ventricles and the ependyma are normal, also the fourth ventricle. Velum interpositum normal. Cross sections of the brain reveal no gross lesions. Cortex is somewhat dark in color. White matter contains an excessive number of blood points. No areas of hemorrhagic encephalitis or softening were discovered. Cross sections of the medulla and pons show no lesions. Both middle ears and the mastoid cells contain an abundant whitish exudate which an examination show Gram positive diplococci apparently in pure culture. The mastoid bone is everywhere hyperaemic.

Cultures of the spleen show streptococci.

Longitudinal sinus and lateral sinuses and upper portion of internal jugular veins are free of thrombi.

The very great prominence of the symptom of lock-jaw coming on early in the disease, together with a history of a recent vaccination, must be our excuse for failing to arrive at the correct diagnosis. The fact, however, that the patient became unconscious at such an early stage led us to doubt the existence of tetanus several days before death, although the true cause of the symptoms was not suspected.

Blood cultures taken at an early period might have led to a correct conclusion and yet there may not have been more than a localized suppurative condition in the mastoid processes until a few days before death.

The case teaches, what is now more generally recognized, that mastoid disease may exist without there being any local evidence of it or even any distinct involvement of the middle ear, and, further, that such a process localized in the mastoid cells with the subsequent occurrence of general sepsis may give rise to a condition of lockjaw, together with additional symptoms not to be distinguished from those ordinarily due to localized lesions in the meninges.

We wish to express our appreciation of Dr. Hill's successful efforts in obtaining permission for a postmortem examination, without which the true nature of the case must have remained more or less conjectural.

FOUR CASES OF TYPHUS FEVER (BRILL'S DISEASE) IN ONE FAMILY

WITH SUCCESSFUL INOCULATION INTO GUINEA-PIGS AND MONKEY

MATHIAS NICOLL, JR., CHARLES KRUMWIEDE, JR., JOSEPHINE S. PRATT, AND JESSE G. M. BULLOWA

The recent work of Anderson and Goldberger of the Hygienic Laboratory at Washington establishing, experimentally, the identity of typhus fever and so-called Brill's disease, together with the mode of transmission of typhus fever by body-lice as shown by these writers, and Nicolle and his co-workers abroad, and by head-lice as lately shown by Anderson and Goldberger, greatly increase the interest in cases of what we must now regard as endemic typhus fever, so well studied by Drs. Brill, Louria and others as it occurs in this country and especially in New York City.

For many months before the occurrence of those reported, no cases of typhus fever had occurred in New York to the best of our knowledge. Those that we present are unique in the history of the endemic form of the disease as it has been studied in New York, in that they affected four members of one family and that two of them were young children. These cases, furthermore, represent types of the disease varying from the comparatively mild, to an example of typhus fever such as is commonly seen in epidemics, thus giving, as it were, a clinical demonstration of the identity of the endemic (Brill's) and epidemic form. But one member of this family escaped the disease, a child of 1½ years of age.

A careful investigation of the premises (a large tenement house) in which these cases occurred failed to reveal the occurrence of any other cases previously, and an extensive inquiry among physicians and hospitals has brought to light but three additional cases of the disease up to the present time.

The patients were typical east-side residents, Russian Hebrews of the poorer class. They had been in the country for about eight months.

REPORTS OF CASES

CASE 1.—Ida K., aged twenty-eight (Chart 1), the mother, was admitted to the Har Moriah Hospital, April 9, 1912, with a temperature of 105° F. She had previously been healthy and there was nothing in her past history to throw any light on her present condition. About six days before admission she had a severe chill followed by fever, sweating and intense headache. She was obliged to take to her bed. Four days before admission she vomited some bilious material. These symptoms continued, together with a profuse watery diarrhea, up to the time of her entrance to the hospital. On admission the patient's condition was fairly favorable; a few hemorrhagic spots were noted on the abdomen. The general physical examination, together with two Widal tests, proved

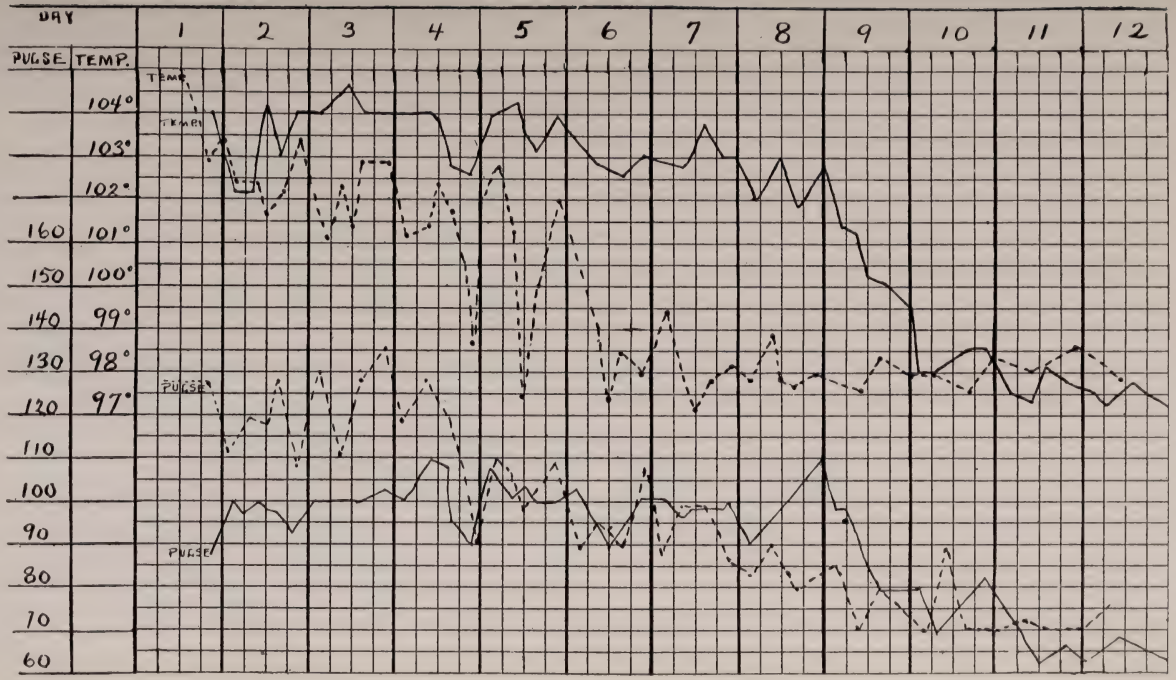


CHART 1.—Pulse and temperature in cases of Israel K. (solid line) and Ida K. (line composed of dashes).

negative. On April 10, the leukocytes were 12,600 per c. mm. of which 66 per cent. were polynuclears. A second count on April 13 showed 11,200 leukocytes and 64 per cent. polynuclears. The case was regarded as one of intestinal toxemia, the true cause not being suspected until after the entrance to the same hospital of other members of the family.

Case 2.—Israel K. (Chart 1), the husband of Ida K., was admitted to the hospital May 4. There was nothing in his personal history of importance except that the patient had suffered for some ten years with a functional gastric condition, but had otherwise been in good health. He used alcohol moderately and was a heavy smoker. Six days before admission to the hospital he went to bed feeling very ill with severe frontal headache and fever. He had a slight cough. He was treated symptomatically by a private physician until his admission to the hospital, where he was seen by two of us three days later and showed the following picture: The entire body from the neck down was covered with a discrete, somewhat morbilliform type of eruption, which included also the palms of the hands. The lesions were fairly widely separated, much more so than in measles, were of bluish-red color, and disappeared on deep pressure. The throat was intensely red, the head somewhat retracted, the neck stiff, and Kernig's and Babinski's signs were marked. The patient's picture was that of typical coma-vigil, the eyes wide open and staring. There was no resistance or evidence of pain caused by taking blood from the arm for purposes of inoculation. The heavy beard, axillæ, scalp and chest were full of lice. Blood-count, May 5, showed 17,000 leukocytes of which 84 per cent. were polynucleated; May 7, 16,000 leukocytes, 80 per cent. polynuclears. The patient was transferred to Willard Parker Hospital, May 11. A physical examination at this time showed the eruption still present with the addition of some petechiæ. The throat was moderately congested, the tongue dry and covered with sordes, as were also the teeth; the conjunctivæ were injected. The patient was emaciated. The lungs showed the presence of bronchitis of moderate extent; the liver was normal, the spleen enlarged and tender, the urine negative. May 12, the patient still coughed and the pulse was very weak. May 15, the patient had greatly improved with the fall of temperature; the rash was still visible and the pulse occasionally weak. Apart from a rather long-continued subnormal temperature his recovery was uneventful. Three Widals proved negative and the stools showed no typhoid bacilli.

Case 3.—Harry K. (Chart 2), aged eight years, was admitted to the same hospital May 8, having been taken ill three days previously with headache, fever and vomiting. The blood-count, May 8, showed 16,400 leukocytes, 82 per cent. polynuclears. Seen on the second day after admission, the patient did not seem particularly ill; there was a maculopapular rash over the body and extremities, the number of lesions being few and widely scattered. They were of the same general type as those seen on the father but much less marked. A physical examination on the day of his transfer to the Willard Parker Hospital, May 11, showed

a fairly well-developed child with hair full of vermin, flushed face and somewhat apathetic. The rash was still present but fading. The lungs, heart and liver were negative, the spleen was enlarged and somewhat tender. An examination of the spinal fluid gave the following results: 23 c. c. clear fluid, pressure considerably increased. Cytology: no cellular elements—coarse shreds of fibrin. There were no bacteria in either smear or culture. Albumin ++. Globulin +. The progress of this case was uneventful to recovery. The pulse after the fall in temperature was frequently quite weak and irregular. The patient had a slight cough for a few days.

Case 4.—Lena K. (Chart 2), aged ten, was admitted to the hospital on the same day as her brother, having been taken ill four days previously with headache, fever and loss of appetite. A blood examination showed: the leukocytes 12,400, polynuclears 76 per cent. When seen on the day following admission the patient looked very ill, frequently groaning as though in pain and with irritability alternating with apathy. Her condition markedly resembled typhoid fever at its height. Her headache was intense, and the rash was similar in appearance and extent to that of her brother. A physical examination, May 15, showed no abnormality other than a somewhat enlarged and tender spleen. The rash was fading and the patient had a slight cough. It was noted that her head was full of vermin. She made an uneventful recovery without other symptoms than those which usually accompany an infectious fever. Examination of the spinal fluid gave the following results: 8 c. c. of bloody fluid under very light pressure. Cytology: many red blood-cells, few leukocytes and polymorphonuclears; also brown crystals. There were no bacteria in either smear or culture. Albumin +. Globulin +.

The blood examination of these patients gave the results noted in the histories. In the father and two children the polymorphonuclear leukocytes showed the inclusion bodies described by Döhle and others as occurring in scarlet fever and other diseases. The mother's blood was not examined for this condition. The treatment was purely symptomatic. Measures against the spread of this disease to physicians and nurses consisted in shaving the hair and the free use of parasiticides; the clothes were disinfected by steam.

We believe that there can be no reasonable doubt that these four patients were infected with the same virus, that of typhus fever.

Results of Inoculations

Israel K.: Blood was drawn May 9, and 2 c. c. directly injected intraperitoneally into Guinea-Pigs 462 and 468 at the bedside. The remaining 4 c. c. were partially defibrinated. At the laboratory the clot was broken up in citrate solution and this added to the defibrinated blood and injected into Monkey A. This monkey at the same time received 1 c. c. of citrated blood from the next patient, Lena K.

Result: Monkey A. On the eleventh day the temperature rose to between 104° and 105° F. for twenty-four hours. This may or may not have been an abortive reaction, which, however, could be proved only by showing that the monkey is or is not immune.

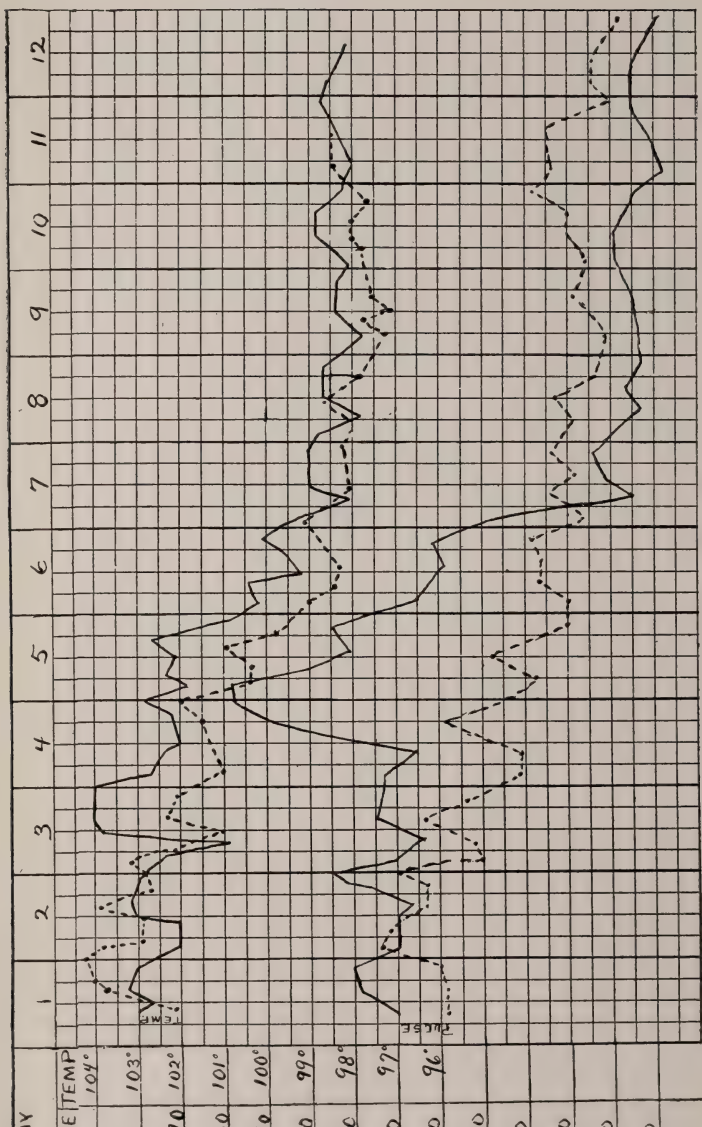


Chart 2.—Pulse and temperature in cases of Harry K. (solid line) and Lena K. (line composed of dashes).

Guinea-Pig 462: Positive. There was a definite rising temperature from the fifteenth to the twenty-first day when the animal died as a result of bleeding from the heart.

Guinea-Pig 468: Positive. Except for a transient rise on the twelfth day there was a definite rise starting on the fifteenth day.

Although the inoculation of Monkey A was apparently negative, the results in the guinea-pigs must be considered positive as evidenced by the reaction of the monkey inoculated with their blood.

Monkey E, inoculated with blood from Guinea-Pigs 462 and 468. A chart of the temperature showed a typical typhus temperature curve. Examination of blood-smears on the twelfth day showed the presence of the same inclusion bodies in the leukocytes as were found in the clinical cases.

Lena K.: May 9, 1912, 1 c.c. of citrated blood was injected into Monkey A, as already mentioned. On the next day, May 10, 10 c.c. of defibrinated blood was obtained and injected into Monkey B. May 11, 3 c.c. of cerebrospinal fluid was injected into Guinea-Pig 437.

Results: All the animals were negative.

Harry K.: May 11, 3 c.c. of cerebrospinal fluid was injected into Guinea-Pig 423.

Results: Negative.

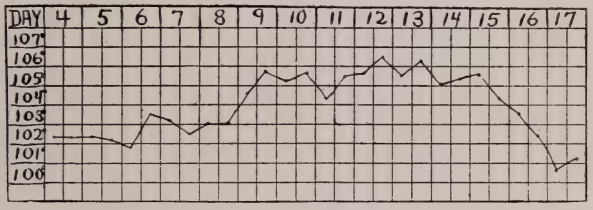


Chart 3.—Temperature of Monkey E.

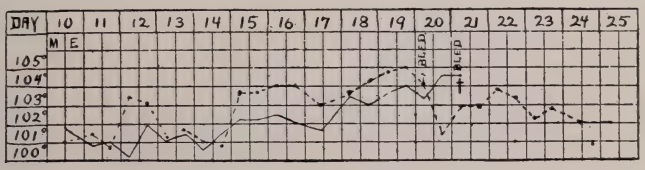


Chart 4.—Temperature of Guinea-Pigs 462 (solid line) and 468 (line of dashes).

One case, therefore, that of Israel K., gave positive results in two guinea-pigs whose blood, injected into a monkey, caused a typical typhus temperature reaction. This, then, is a verification of the inoculability of so-called "Brill's disease" or endemic typhus into monkeys. It also shows that it is possible to infect guinea-pigs directly with this disease, as has been shown in the case of typhus fever by Nicolle, Anderson and Goldberger. This, as far as we know, is the first time that the endemic type

of typhus as it occurs in New York has been directly transmitted to guinea-pigs, and is an additional point of similarity to the epidemic type as it occurs elsewhere.

The negative results following the inoculation of cerebrospinal fluid was to be expected as previous workers have not found it infective.

The negative results of inoculation in the case of Lena K. are probably due to the variation in susceptibility of the animals. The doubtful reaction in Monkey A is attributed to the same cause.

We wish to express our thanks to Dr. Leo Steiglitz, attending physician to the Har Moriah Hospital, on whose service the cases occurred, for permission to study and report these cases, and to Dr. H. Steinmetz, the house physician, for his aid.

Note.—Since the above was written it has been brought to our attention that Dr. Warren Coleman of New York City reported at a meeting of the Academy of Medicine, Feb. 15, 1910, four cases of so-called Brill's disease in one family. No details were given.

THE TREATMENT OF FOUR SEVERE GENERALIZED STREPTOCOCCUS INFECTIONS WITH THE COMBINED EMPLOYMENT OF ANTISTREPTOCOCCUS SERUM AND AUTOGENOUS VACCINES

By Nathaniel Bowditch Potter, M. D.

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When first employing vaccine treatment in streptococcus septicaemias, the writer was impressed with the apparently enhanced value of vaccines where one or several doses of an antistreptococcus serum had preceded their administration. The four cases reported in some detail below were given intravenously two or more doses of serum from a horse actively immunized to several varieties of virulent streptococci and subsequently (except in case 2), subcutaneously a series of injections of autogenous vaccines. The writer's hearty thanks are gratefully tendered to the Research Laboratory of the New York City Department of Health for this serum, for some of the vaccines, and for advice in their use. Before describing the first case, the writer desires to repeat the caution he emphasized in two previous papers* that the apparent success of any modern treatment in this infection must always be judged side by side with the results dependent upon a patient's natural resistance and only aided by symptomatic and supporting treatment. This was well illustrated by a serious streptococcus septicaemia, secondary to angina, where the writer was impelled to delay the administration of an autogenous vaccine because at first the high virulence of the organism isolated from this patient's blood prevented any phagocytosis in the opsonic technique, and because later the delay in subculture coincided with a very slight clinical improvement in the patient's condition; and so finally without the assistance of vaccines the patient completely recovered aided by surgery, general measures, and his natural resistance to infection.

Case No. 1. Streptococcus Septicaemia, secondary to an Angina and complicated by Lymphangitis, Pneumonia, Pleurisy and Phlebitis; treated with Antistreptococcus Serum, and Autogenous Vaccines; Recovery.

W. I. F. 38. First seen January 25, 1913, with Dr. E. H. Pool, to whom I am indebted for some of these notes and for the privilege of reporting the case. Dr. J. F. McKernon furnished the first part of the following history: Illness began January 11, with sore throat, difficulty in swallowing, headache, prostration, muscular pains, fever, and occluded nares producing dyspnoea; the entire mucosa of throat congested, dry, and glazed, but without exudate, that of nose, swollen and accompanied by a profuse discharge; cultures from each showed streptococci in pure culture; under appropriate local and general treatment there was some improvement, first of the nasal and then of the pharyngeal mucosa and of the general condition until January 16, when patient complained of an intense headache, severe chill, temperature rose to 104.2; pulse to 120 and

* The Journal of Infectious Diseases, Vol. 4, Nov., 1907; the Journal A. M. A., Nov. 30, 1907; also quoted by W. J. Stone, the Journal A. M. A., Feb. 15, 1913.

respirations to 25 (see chart). During next few days patient's nose and throat cleared up decidedly and her general condition showed some improvement until January 23, when severe pains in legs, in epigastrium, and in lower abdomen, nausea, considerable vomiting of bile stained fluid and a heightened course of fever, increased the gravity of her condition. On the evening of January 24, patient complained of rather severe epigastric pain which persisted with remissions through the night. On January 25, physical examination showed slight dullness and diminished breathing over right base behind; tenderness with very slight muscular defense over the upper right quadrant, considerable tenderness over rest of abdomen, and very pronounced tenderness of leg muscles. That afternoon patient had profuse sweats and in evening a severe and prolonged chill. The leucocyte count was 20,000 with 88 per cent. of polynuclears. A blood culture (Dr. Bradbury) showed a pure culture of streptococcus hemolyticus (see Dr. Krumwiede's report below), each plate being riddled with myriads of colonies. During next day repeated chills, sweats, persistent pains in legs, abdominal pain and tenderness, nausea and vomiting.

Treatment: Pyramidon gr. ii, for temperature above 102°. January 27th sore throat, pain in back of upper left chest without pleural or pulmonary signs to explain it, sweating profusely. 100 c. c. antistreptococcus serum (New York Health Dept.) were slowly injected intravenously in left arm. This was diluted with about 200 c. c. saline and given at a temperature of 105°. Beyond some sweating, and a temporary stimulation of the pulse no immediate effect was noted. January 28, wretched, sleepless night, nausea, no effect of serum noted. January 29th, 75 c. c. of the same serum diluted with 150 c. c. saline were introduced and this time it was found necessary to cut down on the vein. Over right lower chest behind, on this day, there were noted dullness, a few medium moist and many crepitant râles, increased voice and bronchial expiration, as well as slight dullness and a few fine râles at extreme left base near spine; there was also considerable abdominal distention. January 30, pain and stiffness upper left arm, above site of transfusion, dullness at right base increased in intensity and extent, breathing diminished, few crepitations. Pain, tenderness, slight swellng and redness of metacarpo-phalangeal articulation middle finger of right hand. Pain and tenderness in the depths of upper left arm; less abdominal tenderness and distention, temperature intermittent and higher; sweats, chills in a. m. and p. m., nausea and vomiting. The skin over an area of about 3 inches in diameter about and above incision of left arm, red, edematous, and very sensitive, presenting the clinical features of the lymphangitis of erysipelas, dressed at incision with same serum; and above, with acetate of aluminum. January 31. Two chills, some sweating; nausea and vomiting persist. 50 c. c. serum diluted with 100 c. c. saline given intravenously. February 1. Awakened with chill, nausea, weakness, nervousness; pain in left upper chest, crepitations, slight dullness, broncho-vesicular breathing over small area below left clavicle. February 2. Cough with mucoid sputum, sweating. At the right base dullness, diminished breathing and voice with distant bronchial expiration have spread upward to angle of scapula and are

still more striking. Autogenous vaccine (25 Million) injected subcutaneously, chilly but no real chill. Paracentesis of right lower chest below angle of scapula; 100 c. c. sero-fibrinous fluid obtained; coughing and dyspnoea prevented more complete removal; culture of fluid was negative. February 4. Pain in right side below costal margin and in right loin persists; still sweating. 50 Million vaccine injected. February 5. Severe generalized itching, large blotches and purpura, and some erythema nearly covering entire trunk and spreading to extremities. February 6. Signs in right chest same as before tapping; tapped again but only a few ounces sero-fibrinous fluid obtained (culture negative). Exploratory puncture made in right perirenal region on account of persistent pain there; only blood obtained (culture positive). Severe itching, generalized over body. Almost entire body covered with erythema and purpura. February 7. Signs in left upper chest practically disappeared; in extreme left lower, less, in right lower, same. Voice is husky; eruption profuse but purpura less vivid in color. 75 M. vaccine injected. February 8. Stiffness of neck and some pain in moving it. February 9. Blood coagulation time less than two minutes; some diffculty in swallowing, eruption less intense. February 11. Chill. February 12. 100 M. vaccine Painful area on right side below costal margin and extending to back, sensitive to pressure. February 13. Right pleura tapped again, only few ounces sero-fibrinous fluid obtained, bloody at end of tapping; first portion, culture negative; bloody fluid, positive. February 14. 125 M. vaccine. February 17. 150 M. vaccine. February 18. Right leg swollen, tender, painful (phlebitis). February 20. 150 M. vaccine. February 21 Pain in right anterior chest and axilla (friction rub), chest strapped; left leg swollen below knee, painful (phlebitis). February 24. 150 M. vaccine. February 28. Pain and stiffness of neck. March 1. Pain in shoulder joints, 150 M. vaccine. March 4. Severe pain in back of neck; puffiness about eyes. 150 M. vaccine. March 6. Red swollen areas on left arm and on calf of right leg; wet dressing. Paracentesis of right base; 280 c. c. sero-fibrinous fluid (culture negative). March 8. 184 M. vaccine. March 9. Abdominal distention. March 11. Despite stupes enemas and irrigations, abdominal distention has increased strikingly; patient in great pain; pain paroxysmal; vomiting at first of bile stained fluid; later of brownish and offensive slimy and thicker fluid; symptoms of almost complete obstruction occurred; seen by Drs. Brewer and S. W. Lambert. Obstruction finally overcome by irrigation. A similar but less severe attack occurred, toward the end of March and again in middle of April. Except for edema of legs and occasional attacks of pain in upper right quadrant of abdomen, associated with moderate abdominal distention, patient's convalescence proceeded uninterruptedly. During October, one of these attacks of pain was followed by complete obstructive jaundice; operation was performed by Dr. Pool and a large stone was removed from common duct and several from the gall bladder. On the second day after the operation, there began persistent oozing which continued, in spite of all the usual measures for its control. Three transfusions made by Dr Linderman finally proved effective. At the time of writing, six weeks

after operation the patient is in excellent condition and the wound is practically healed.

Bacteriological Report (Dr. Krumwiede)

Organism isolated from two blood cultures and from one specimen of serum from pleura quite deeply stained with blood. A typical streptococcus, growing in chains of 10 to 20 individuals in fluid media, the individual cocci being large and somewhat irregular. On blood agar the colonies are surrounded by an unusually large zone of haemolysis. The organism is a typical *streptococcus hemolyticus.* This, a very severe bacteriaemia secondary to an angina, showed relatively little natural resistance even early in the attack. The lymphatic glands draining the infected throat were scarcely enlarged. Her prognosis as judged by her clinical picture was for several weeks very grave and during the first attack of intestinal obstruction practically hopeless. The blood culture was strongly positive early in her attack and remained positive for a long time. The leucocytes showed at first a moderately high count with a high percentage of polynuclears cells, but promptly following the inoculation of the protective serum there was a sharp fall in both high cell count and the polynuclear percentage, and these remained low throughout the rest of her illness. The hemoglobin, on the contrary, was well sustained throughout the infection. The urine gave evidence of severe intoxication throughout most of her febrile course, but eventually cleared up. Although there were evidently many depots of infection scattered throughout the body and frequent and varied complications, the endocardium never became involved. The value of the protective serum in this case is not easy to judge. It was evidently insufficient to do more than assist the patient to hold her own, and scarcely any general improvement was noted until after the employment of the vaccines. Their use certainly suggested very strongly a definite curative influence, although the statements of such profound students of infection as Theobald Smith, Pearce and others question definite therapeutic value of vaccines in generalized infections. In this patient the serum rash was the most profound and distressing which the writer has ever witnessed. As far as its dosage and intervals for its employment are concerned, in future one would be tempted to employ larger doses at shorter intervals and then desist if no improvement was noted.

Case 2. *Streptococcus Septicaemia following a Septic Hand and Forearm with Lymphangitis, treated by incisions and with Antistreptococcus Serum and Vaccines, Recovery.*

Male. 38. Seen March 30, with Dr. Hanna, to whom I am indebted for the privilege of reporting the case. Patient was admitted to the French Hospital, March 13, 1913, No. 7544, with a septic hand and forearm and slight lymphangitis. The next day numerous deep incisions were made, some pus evacuated, and saline dressings applied; and a mixed stock vaccine (25 Million Streptococci, 100 M. Staphylococci, and 50 M. pneumococci) were injected subcutaneously. Subsequent incisions on the 17th and 19th; the same dose of vaccine repeated on the 18th and Bier's

cups applied for several days. On the 28th patient had a chill and a sudden rise in temperature; on the 29th a blood culture revealed numerous colonies of a short chained streptococcus hemolyticus (Dr. Krumwiede); and a third dose of same vaccine was given. On the 30th at the writer's suggestion 100 c. c. of an antistreptococcus serum well diluted with normal saline was given intravenously; and the wounds were irrigated with the serum and packed with gauze soaked in the serum. On April 1, the white cells were 22,000, with 86 per cent. polys; and the serum was repeated. From the onset of the fever on the 28th the patient's condition was grave, with profound weakness, rapid feeble pulse, dusky gray color, apathy, stupor or delirium, urine and feces often involuntary. The wounds began to improve within 48 hours after the employment (local and intravenous) of the serum; the second blood culture, taken 6 days after the first and 4 days after the second dose of serum, was sterile; but there was no striking general improvement until several days after the second dose of serum. The serum rash was slight. His convalescence was slow but recovery practically complete.

This, a very severe but localized streptococcus infection with marked constitutional disturbance, became a generalized bacteriaemia with profound sepsis and that despite repeated incisions and apparently excellent drainage. The value of the serum applied locally was unquestionable, but no such claim can be made for its intravenous use. The leucocytes and polynuclear percentage remained high two days after the first dose of serum. The improvement of the hand and arm was no greater than could be accounted for by the topical application. The second blood culture, it is true, was negative; but the general clinical condition improved too slowly, to justify attributing the recovery to the intravenous serum. No autogenous vaccine was employed in this case.

Case 3. *Streptococcus Septicemia following an operation upon Acute Cervical Lymphadenitis secondary to an Angina and complicated by Multiple Arthritis, including a Purulent Monarticular Arthritis; treated with Antistreptococcus Serum and Autogenous Vaccines; and numerous Aspirations of Purulent Joint; Recovery.*

Seen with Drs. J. A. Blake and Fraenkel to whom I am indebted for the privilege of including this case in my report.

T. K. 21, male, onset 8 days before entrance (May 12, 1913), to Presbyterian Hospital: with sore throat, tender and enlarged cervical glands on both sides, throat culture showed no diphtheria bacilli, chill, headache, fever, 101° to 103° for 3 days, then none for 2 days, gradually rising since; diphtheria antitoxin 6 days before entrance, sore throat disappeared within 3 days, right cervical glands subsided, but those of left side steadily enlarged until incised by Dr. Blake, May 10th, 2 days before entrance; an ounce of pus evacuated; fever about 105°, patient says he has had a chill nearly every day, but no sweats, vomited day before entrance, anorexia.

May 11th. Leucocytes 14,000, polys. 87 per cent.

May 12th. Leucocytes 22,000, polys. 85 per cent.; 28,800, polys. 81 per cent.; 32,700, polys. 80 per cent.; chill; blood culture showed a "streptococcus probably a hemolyticus."

May 13th. Chill both morning and afternoon.

May 14th. Another chill. 200 c. c. antistreptococcus horse serum (N. Y. Health Dept.) were given intravenously very slowly and well diluted with saline. No untoward effect and no appreciable benefit noted.

May 15th. Leucocytes 19,000, polys. 79 per cent. Condition unchanged.

May 16th. Leucocytes 15,500, polys. 78 per cent.; 25 Millions autogenous vaccine given subcutaneously.

May 17th. Leucocytes 10,800, polys. 72 per cent.

May 18th. Leucocytes, 9,900, polys. 72 per cent.; occasional vomiting, 50 M. vaccine.

May 19th. Leucocytes 9,900, polys. 73 per cent.; Hgb.=90 per cent.; Reds=4,040,000, spleen palpable, chill.

May 20th. Condition unchanged, leucocytes 11,800, polys. 72 per cent.; blood culture positive.

May 21st. Profuse serum rash, neck wound healed. Leucocytes 11,600, polys. 74 per cent.

May 22nd. Chill, 100 M. vaccine.

May 23rd. Blood culture positive.

May 25th. Chill, 170 c. c. serum from another horse immunized to streptococcus veridans.

A very profound and alarming general reaction followed with symptoms of dyspnoea and collapse. The reason for the change in serum was the failure of any notable benefit from the two previous doses and the later bacteriological report that the organism was one intermediate in form between veridans and hemolyticus.

May 26th. Chill. Leucocytes 6,900, polys. 76 per cent.

May 27th. Hgb. 80 per cent.; Reds 4,132,000, 200 M. vaccine.

May 29th. Vaccine 200 M. (living).

May 30th. Transported to seashore and kept out of doors.

May 31st. Leucocytes 16,800, polys. 80 per cent.

June 1st. Leucocytes 13,600, polys. 74 per cent. Eosinophiles 3 per cent., pain and swelling in right knee.

June 2nd. 400 M. vaccine.

June 4th. Chill, leucocytes 10,200, polys. 83 per cent.; urine normal.

June 5th. Left knee, finger and toes painful.

June 6th. 100 c. c. cloudy fluid aspirated from right knee, culture positive.

June 8th. Chills morning and afternoon, leucocytes 18,800, polys. 85 per cent., Hgb.=70 %.

June 10th. 30 c. c. pus aspirated from right knee. Fingers and toes better. The subsequent course is well shown in the chart. The aspirations were frequently repeated and the autogenous living vaccine continued in increasing doses until July 10th, when a severe reaction followed and the dose was diminished and the interval lengthened. The right foot became swollen on June 29th but subsided shortly. The lowest Hemoglobin was 64 per cent. on July 10th. From that time it began to rise. The patient's recovery is complete.

Bacteriological Report (Dr. Cecil)

This organism, isolated in pure culture from three different blood cultures, and three specimens of exudate from knee joint showed the following characteristics:

A gram positive coccus of fairly good size growing in short and long chains, often in the form of diplococci, lanceolate or biscuit shaped. No capsule demonstrated.

Cultural characters:

Blood broth: Cloudy, slight haemolysis after five or six days growth.

Blood agar: No definite haemolysis. Faint suggestion of green along streaks, where growth shows itself in the form of a delicate greyish growth.

Glucose agar: Delicate greyish colonies.

Ascitic agar: Growth more luxuriant. Moderate amount of precipitation.

Plain broth: Slightly cloudy, with sediment.

Inulin: Not coagulated.

Bile Test: Negative.

The organism is a streptococcus intermediate in form between viridans and haemolyticus.

This, a very severe and prolonged bacteriaemia, resulting from the same clinical type of infection as in Cases 1 and 3, but produced by an organism distinct from all the other three cases, occurred in a youth of vigorous resistance. After the bacteriological studies were completed, it became evident that neither serum employed in the treatment could be called in any way specific; nor can we claim any clinical improvement from their use. Fortunately the endocardium was not attacked; but many joints were more or less severely affected and one of them became purulent. Recovery was evidently due to the patient's sturdy natural resistance, stimulated by ideal surroundings and apparently aided by the vaccines; but especially to the repeated aspirations of the purulent joint. The first doses of living vaccine employed were prepared from the organism isolated from the patient's blood and were sensitized by the same serum as was used intravenously May 14th and 19th; the later doses were prepared from the organism isolated from the purulent knee and they were sensitized by the serum from rabbits treated with joint organism. It was remarkable what large doses of living vaccine the patient was able to take without the production of a lesion at the site of inoculation or any evidence of a general reaction until July 10th, when 2,000 Million were administered.

Case 4. *Streptococcus Septicemia, secondary to an Angina and complicated by Adenitis, Pneumonia, Pleurisy, Arthritis, Decubitus, Abscesses, treated with Antistreptococcus Serum and Autogenous Vaccines; Recovery.*

Male, Irish, age 45, laborer, entered New York City Hospital May 13, 1913.

Complaint: Cough, pain in chest and left side of neck.

History (obtained from patient since recovery): Measles in child-

hood. Venereal diseases denied. Two years ago an inflammation in the left side of neck which gradually disappeared. No other illness that he remembers. Alcohol and tobacco moderate. For the past week patient has had a feeling of general malaise, what he called "Grip," and a sore throat. Three days ago he had a chill lasting about an hour accompanied by fever, a distressing cough with some yellowish expectoration, and severe pain and swelling in the left side of the neck above the clavicle. Pain was also present in the left lower chest. Went to bed at his lodging house and remembered nothing more until he regained consciousness some days after entrance to City Hospital.

Physical Examination on Entrance. Well developed and fairly well nourished male, lies in dorsal decubitis breathing rapidly and with marked difficulty, face is flushed, skin hot and dry. Mentality, confused, delirious or stuporous but can be aroused to respond to questions, although his answers are irrational.

Eyes: Pupils, conjunctivae, sclerae normal. Nose and ears normal.

Mouth: Mucous membrane slightly cyanotic, dry. Tongue covered with a heavy yellowish white coat, teeth poor, pharynx congested, yellowish gray patch on left tonsil size of a large pea surrounded by congested mucous membrane. Skin over left anterior surface of neck above clavicle is hot, red, infiltrated and evidently sensitive to touch, beneath it can be felt a number of enlarged and very tender lymph glands matted together, and obscured by extensive subcutaneous and deep edema. No fluctuation. Post cervical glands on this side are moderately enlarged.

Chest: Expansion limited to the right side, left side appears fixed, respirations are rapid and labored.

Lungs: Percussion note dull at the left lower chest anteriorly; and posteriorly nearly flat; the breath sounds in the latter region are distant. Over the left upper lobe many coarse râles, bronchial breathing, and increased vocal fremitus; over right lower crepitant and sub-crepitant râles, and harsh breathing.

Heart: Neither thrills, nor palpable apex beat; no enlargement determined, action rapid but sounds of good quality.

Pulse: Regular, rapid, poor quality, artery not palpable.

Systolic Blood Pressure: 100 m. m. Hg.

Liver: Reaches from the 6th rib to costal margin.

Treatment: Camphor grs. 1 q. 2 h. by hypodermic. Digipuratum m. X q.. 3 h. Alcohol sponge q. 4 h. if temperature over 103°.

May 14. Excursion still limited on left. Dullness in right axilliary region and in right back from angle of scapula down, flat at extreme base, where the breath sounds are scarcely audible, vocal fremitus diminished, and tactile fremitus absent. Rhonchi are heard constantly throughout the right chest. Left supraclavicular region is still swollen red, and tender. *Leucocytes* 9,600, Polys. 92 per cent, no eosinophiles.

Sputum yellowish green, cocci, many in pairs; no tubercle bacilli. Patient was troubled throughout the day by constant coughing; and during the night became more irrational and required restraint.

Treatment: Whiskey ½ ℥. q. 4 h. Camphor grs. 1 q. 3 h. Digipuratum discontinued.

May 15. Signs at left apex clearing also less marked over left lower lobe. Slight swelling and edema about left wrist, and back of hand. Patch on left tonsil grayish. Smear shows numerous cocci in pairs; no other organisms noted. Patient remained irrational all day, appeared weak at night. He was quieter during the night, slept very little; but took nourishment well. Camphor discontinued. Leucocytes 14,000, Polys. 81 per cent., no eosinophiles. Blood culture taken.

May 16. Signs over both lobes of left chest practically cleared. Paracentesis of right lower chest. No fluid or pus obtained. Throughout the day, patient remained irrational, was incontinent of feces and urine.

Treatment: Digalen M X q. 4 h.

May 17. Patient incontinent, looks more septic; abdomen markedly distended; continued irrational; toward night appeared weaker. Coughing troublesome, rather productive and sputum streaked with blood for the first time. Dullness to flatness from angles of right scapula down, moderately fine friction rub, and medium and coarse moist râles, no dislocation of heart.

May 18. Patient still incontinent, less coughing, appears weaker.

May 19. Blood culture taken 15th reported to contain *Streptococci.*

Throat culture taken 15th reported to contain Staphylococci. Paracentesis of right chest repeated; about 15 c. c. of turbid fluid, either pus or fibrin (thrown away by mistake). Patient still incontinent. 9 p. m. Venesection 120 c. c. of blood removed from vein in left arm and very slowly replaced by 200 c. c. of antistreptococcus horse serum (N. Y. Health Dept.) in 400 c. c. of normal saline. Character of the pulse improved and the patient showed no ill effects either during or after the infusion. Blood Pressure rose 10 m. m. Hg.

May 20. Patient's general appearance strikingly improved, appears rational, talks naturally and says he feels better.

May 21. Patient does not appear as well, pulse weaker. Two areas, each about the size of a 50 cent piece, appeared beneath skin on tip of each heel. They were dark blue in color; were not tender nor were they surrounded by any inflammatory zone or lymphangitis. Patient placed in a water bed. Profuse sweat three times during the day. Does not expectorate much but disturbed by coughing. Takes nourishment fairly well. Whiskey 3 ii; q. 4 h. Diagalen m. vii b. i. d.

May 22. Slight improvement in general condition, respirations still labored, gradual increase in redness and swelling at left wrist, marked tenderness, slight fluctuation; aspirated but no fluid was obtained. Wet dressing and splint applied. Small pressure sore at the sacrum. Blebs of heels increased in size without signs of active inflammation. 3:30 p. m. 200 c. c. of antistreptococcus serum in 400 c. c. of normal saline given intravenously without untoward effects until 4:00 p. m. when patient had a severe chill lasting 20 minutes and accompanied by a profuse sweat.

Urine Examination: Amber, acid, specific gravity 1018, albumen, trace; sugar absent, sediment, many red and white cells; many hyaline and granular casts.

Leucocytes 18,800; polys. 85 per cent., no eosinophiles. Evening: Patient appears rational; is still incontinent; takes nourishment readily.

May 23. Patient appears distinctly improved and says he feels much better. Fluctuation over left wrist; hand also swollen and movement of either hand or wrist is very painful.

May 24. Patient appears comfortable but does not look as well as yesterday, has regained the septic look which seemed to disappear temporarily after each infusion of serum. Right base is flat on percussion, vocal and tactile fremitus diminished above and absent below, breath sounds absent. Few cracking râles (friction) heard. Breath sounds in axillary region nearly normal. Decubitus gradually increased in size and edges undermined but without signs of active inflammation. P. M., 200 c. c. of antistreptococcus serum with 400 c. c. of normal saline given intravenously, no reaction followed. Patient has perspired freely all day. At site of previous venesection there has developed a small abscess. Opened and dressed with antistreptococcus serum.

May 25. Condition unchanged, coughed less frequently, sweating profuse throughout the day, but very little during the night. Decubitus over sacrum and both heels dressed with antistreptococcus serum.

May 27. Hoarseness; respirations more rapid; left wrist remains about the same except for slight increase in the swelling on palmar surface. 9 p. m. 100 c. c. of antistreptococcus serum given with 200 c. c. of normal saline intravenously. No reaction followed. Leucocytes 15,200; Polys. 88 per cent.

May 28. Condition good. Hoarsness persists. Upper lip swollen. Raised white blotches on back from shoulder to sacral region. The indurated area above left clavicle extends quite deeply. Glands in neck less sharply outlined; slightly more tenderness. Left wrist and dorsum of hand presents no appreciable change except oedema slightly more marked on palmar surface. Right heel decubitus somewhat larger; chest signs unchanged except for slightly more resonant note over right base, breathing still dinimished, distant, and bronchial in character. Crepitations heard mostly at the end of inspiration, some with expiration. Expectoration thick yellowish and sticky, one pledget tinged with blood. Spleen not felt. Pallor. (Hgb.=90 per cent). Blood culture taken (reported negative later). During night patient had an attack of dyspnoea which lasted a few minutes.

May 29. Urticaria is more general in its distribution. Wheals are reddish in color. Lips are more puffy, although patient is not as hoarse nor does he complain so much of throat. Left wrist is not so painful and swelling has subsided to some extent. Less discharge from abscess at site of venesection.

May 30. Condition on both heels shows slight extension. General condition fair. Throat rather congested but otherwise negative. Slight cough, with some expectoration. Urticaria more diffuse covers practically the entire body but pruritus not distressing and color begins to fade. Wrist slightly less swollen and not as tender. 7.30 p. m. 70 c. c. of antistreptococcus serum diluted with normal saline injected into tissues of axilla and abdominal wall. Patient takes nourishment well and sleeps a greater portion of the night.

June 1. General condition unchanged. Fever, pulse rate, and pallor

increased. Hand and wrist slightly less swollen, tenderness unchanged. No tenderness over sternum. Systolic murmur heard most distinctly over the aortic area. Spleen not palpable. Abdomen still somewhat distended. Punched out ulcer in depths of bedsore apparently connects with a deeper tract. There is a broad zone of dullness extending from just below the angle of right scapula with flatness at the base of the lung and at its upper edge the voice sounds are most intense and rather nasal and the breathing distant and bronchial; below this the breathing is feebler and accompanied by fine and medium moist râles. At the left base posteriorly there are numerous moist râles.

June 2. 300 million autogenous streptococcus vaccine injected subcutaneously.*

June 3. Patient complains of pain in the right shoulder. Sweat freely for a short time in p. m.

June 4. Blood culture taken (reported negative later).

June 6. Patient sweats profusely in day time; bedsores improving general condition same, signs in right chest same. Vaccine 600 m.

June 7. Thoracentesis of right chest 2 c. c. sero-fibrinous fluid obtained (culture reported later negative).

June 8. Less sweating. Vaccine 900 m.

June 9. *Leucocytes* 10,400; Polys 78 per cent.; cosinophiles 3 per cent. Hgb. 70 per cent.

June 13. Relatively little change in heels, granulations indolent, covered with a thick pus underneath the skin. About half way down left leg, below the knee oedematous area size of a lemon, thickened, reddened and tender. No enlargement nor tenderness of femoral glands. Neither spleen nor liver palpable. Bedsore smaller, looks healthier.

June 17. Aspiration of abscess in calf of left leg reveals pus with pure culture of streptococcus.

June 19. Abscess of leg incised, 10 ounces of pus evacuated. (Dr. Lyle.)

July 3. A fluctuating mass about the size of an English walnut at the deltoid insertion incised and about 10 c. c. of a thick creamy pus evacuated; otherwise patient's general condition, signs in right chest, wrist, bedsore and heels, progressing favorably.

July 30. Patient goes on excursion in wheel chair.

November 25. Patient is in excellent condition, walking is still interfered with by scars of heels; wrist stiff but under massage is becoming more pliable.

Bacteriological Report (Dr. Larkin):

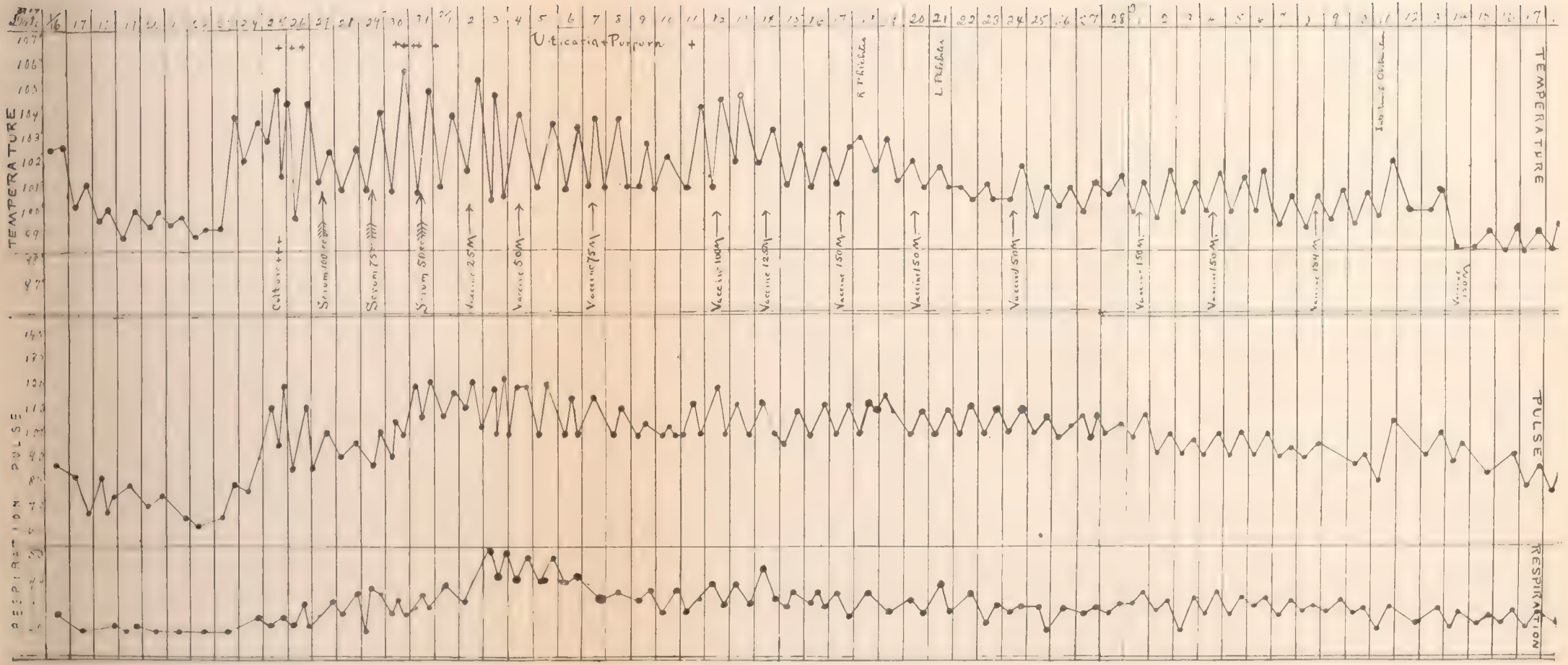
"The organism in pure culture was isolated on several occasions: from two blood cultures (15th and 22d of May), from a bleb of heel (May 29th) and from pus from abscess on leg (June 18th). Blood cultures May 27th and June 4th were negative. Blood agar plates in each instance showed 30—35 colonies with a distinct hemolytic zone surrounding the growth. The broths showed marked hemolysis of the blood. The organism was a Gram positive coccus of a moderate size, but markedly

* The interne who administered this large dose mistook mimins on label for cubic centimeters; it certainly did no harm, and when the mistake was discovered and smaller doses given the patient's progress was not as satisfactory nor as rapid.

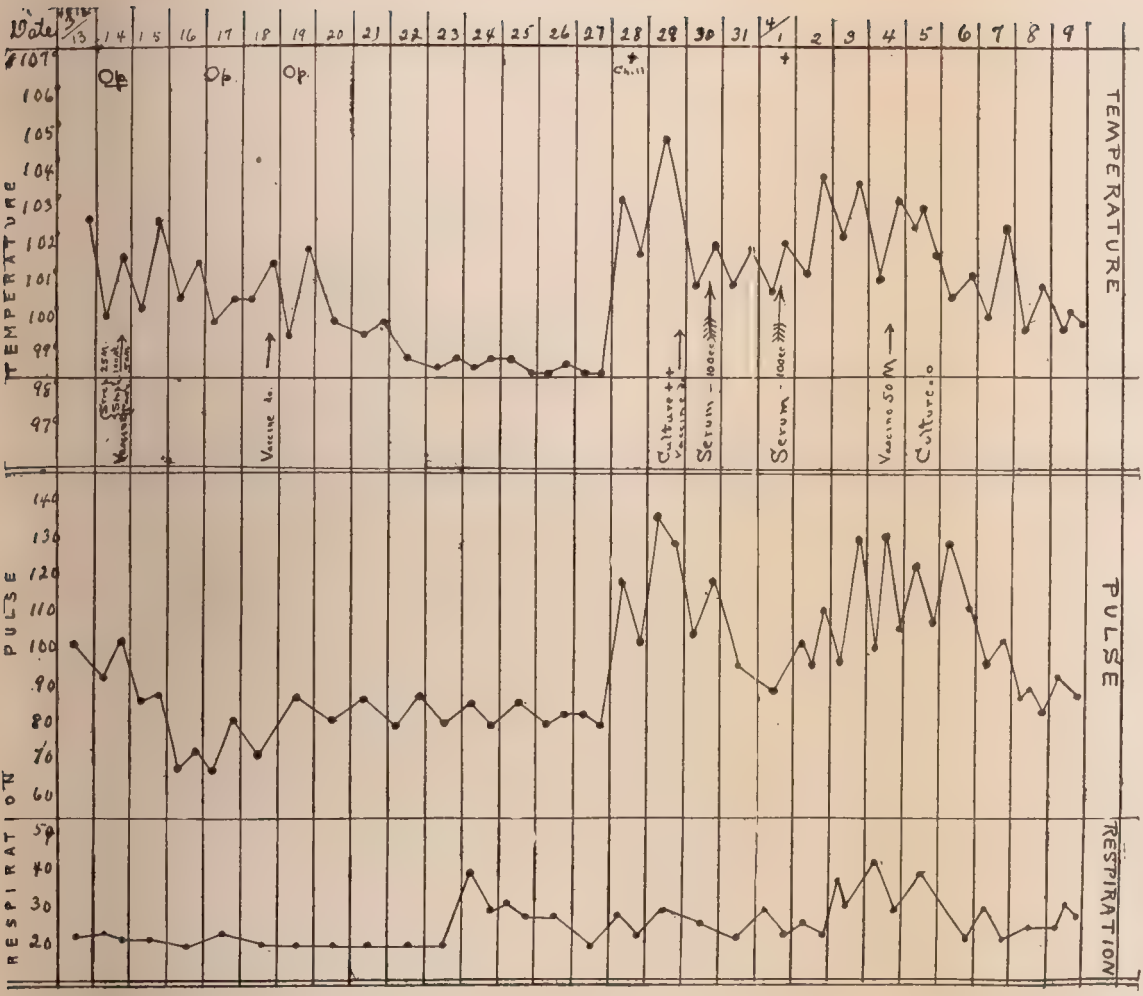
irregular in shape, growing in long chains in fluid media. No capsule was demonstrated. On serum agar the growth was fairly luxuriant with a distinct precipitation after several days' growth. On North media the growth was less marked. Inulin not fermented. Diagnosis: *Streptococcus hemolyticus.*"

From the onset of the infection, this patient was completely overwhelmed. Starting in the throat, the adjoining lymphatic glands and subcutaneous tissues were deeply infiltrated, a large area of lung tissue and a considerable portion of the right pleura were next affected followed shortly by a periarticular involvement of some size and persistency. The spleen was not made out enlarged at any time; and evidently the endocardium was not implicated. The first blood culture was strongly positive; the second, taken just before the serum was introduced, was also strongly positive; the third, nine days after the first does of serum, was negative. The leucocytes which were at first low with a very high percentage of polys, showed a reversal of this ratio before the employment of the serum and more noticeably so afterwards. The haemoglobin never fell below 70 per cent. The urine showed blood and casts but the albumen was never more than a trace, and the formed elements cleared up early in his convalescence. It represents the most severe general streptococcus infection which the writer has seen recover. The description and accompanying chart represent but inadequately the perfectly hopeless prognosis given by the writer and by all of his colleagues (Harlow Brooks, Shelby Camac, Lyle and others) who were interested enough to follow the patient's course. Nor does the chart show the striking clinical improvement which followed each inoculation of serum. The effect of the first dose, at least, might be compared to the result of antitoxin in diphtheria or of the specific serum in epidemic meningitis. Neither the first nor the succeeding doses were sufficient to hold the improvement for more than 24 to 36 hours. Despite the very large dosage of the autogenous vaccines, a distinct clinical improvement apparently followed their use and this improvement did not persist when the dosage was markedly diminished. The serum rash was much less distressing than in the other cases.

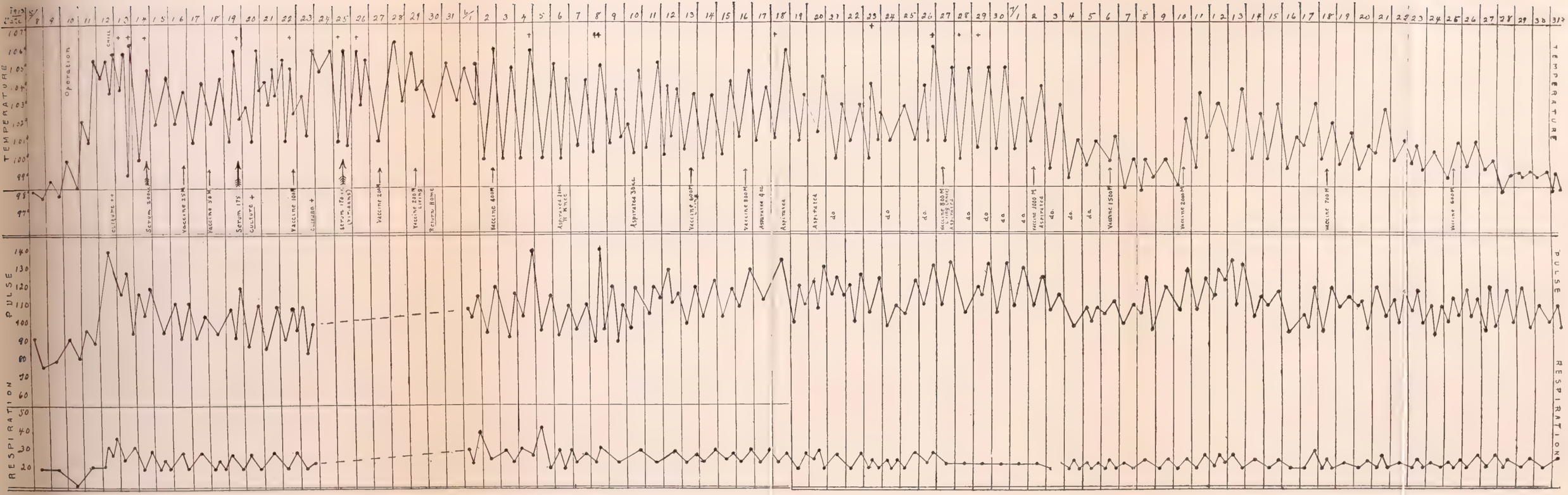
These four cases are reported in this detail in order to stimulate further interest in this method of treatment. They are the only ones which the writer has seen since he has had the opportunity of employing the Health Department's antistreptococcus serum. All four cases recovered and they were all very grave infections. Even if the effect of the serum in all four had been as striking, clinically, as in the last case, no conclusions could be drawn from so few observations; and, in the last case, there is only a strong clinical impression that the serum was of direct and immediate benefit. It seems fair to add another clinical impression which the writer believes is well worth experimental study, and that is, that the value of the vaccines was enhanced in three of these cases by the preceding doses of serum. In three of these cases the local application of the serum was of unquestioned value, and the writer urges its trial, or that of some other protective serum selected in accordance with prompt bacteriologic studies in any severe streptococcus angina.


TEMPERATURE
PULSE
RESPIRATION
Urticaria + Purpura
Culture +++
Serum 100 c.c.
Serum 75
Serum 50
Vaccine 25 M
Vaccine 50 M
Vaccine 75 M
Vaccine 100 M
Vaccine 125 M
Vaccine 150 M
Vaccine 150 M
Vaccine 50 M
Vaccine 150 M
Vaccine 150 M
Vaccine 150 M
R Pleura
L Pleura

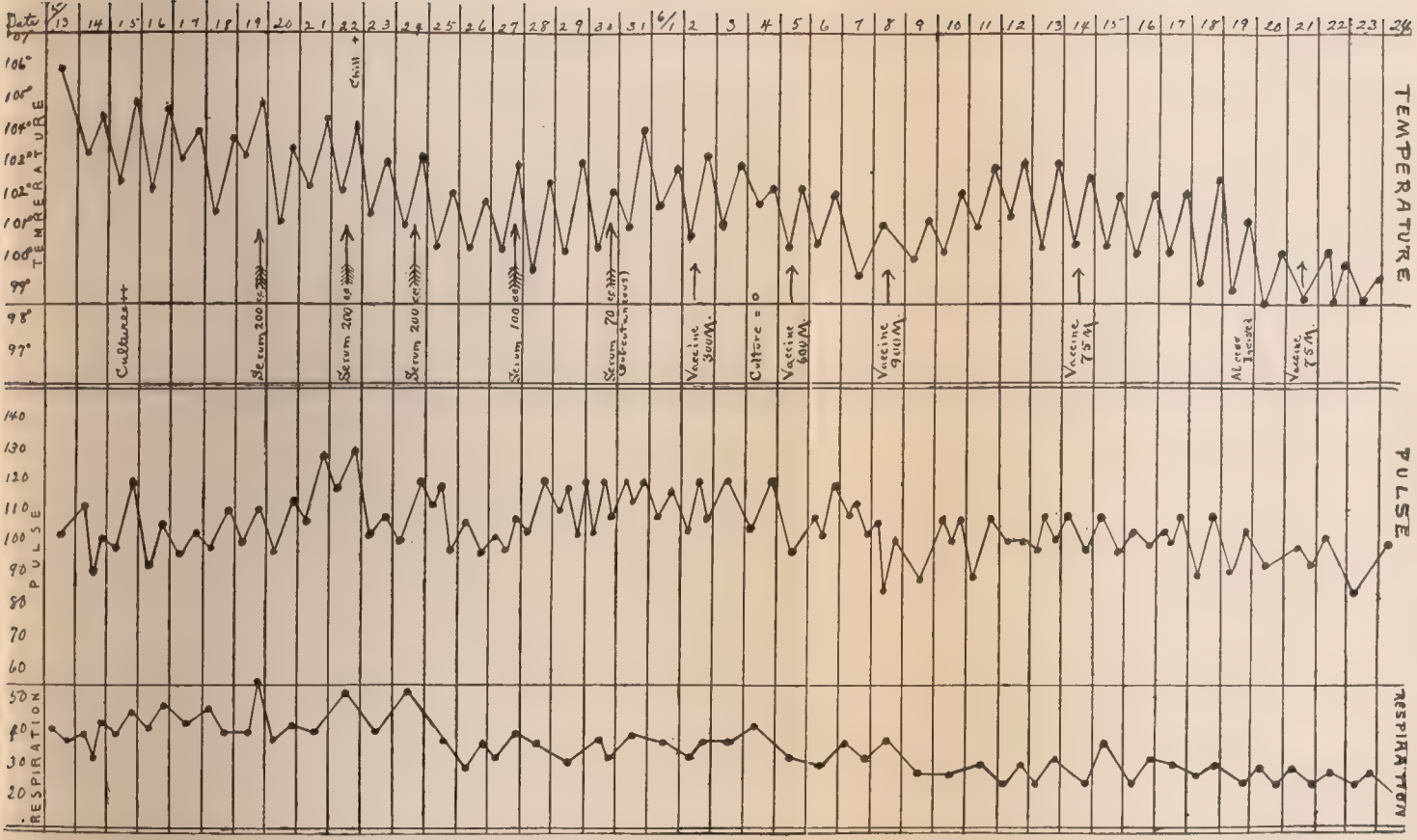
Case No. 1.


Date
TEMPERATURE
PULSE
RESPIRATION
Op.
Op.
Op.
Vaccine 4.
Culture ++
Vaccine 4
Serum - 100 c.c.
Serum - 100 c.c.
Vaccine 50 M
Culture 1.0

Case No. 2.


TEMPERATURE
PULSE
RESPIRATION
Operation
Chill
Culture ++
Serum 200M
Vaccine 25M
Vaccine 50M
Serum 175
Culture +
Vaccine 100M
Culture +
Serum 175cc (Intraneural)
Vaccine 20M
Vaccine 250M Living
Return Home
Vaccine 400M
Aspirated 30cc
Vaccine 600M Living
Vaccine 800M
Aspirated
Vaccine 800M Living Culture
Vaccine 1000M Aspirated
Vaccine 1500M
Vaccine 200M
Vaccine 720M
Vaccine 600M

Case No. 3.


Date
TEMPERATURE
PULSE
RESPIRATION
Culture++
Serum 200 cc
Chill +
Serum 200 cc
Serum 200 cc
Serum 100 cc
Serum 70 cc (Unfortunately)
Vaccine 300 M.
Culture = 0
Vaccine 600 M.
Vaccine 900 M.
Vaccine 75 M.
After Incubel
Vaccine 75 M.

Case No. 4.

A STUDY OF TRACHOMA AND ALLIED CONDITIONS IN THE PUBLIC SCHOOL CHILDREN OF NEW YORK CITY. BY A GROUP OF WORKERS UNDER THE DIRECTION OF ANNA WESSELS WILLIAMS

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I.—INTRODUCTION AND CRITICAL REVIEW OF LITERATURE

By Anna Wessels Williams

Introduction. Judging from the written word our knowledge of trachoma is in a most confused state. This is partly due to the fact that, notwithstanding the voluminous bibliography on the subject no minute critical review of the whole has been made for some time, hence the relations between the older observations and the more recent ones are not clearly defined. For instance, though the majority of opthalmologists consider "Egyptian Ophthalmia" a broad term including several different diseases, such as gonorrheal ophthalmia, acute contagious conjunctivitis ("pink eye"), and trachoma, some ophthalmologists still use the term

as synonymous with trachoma only. Again, though the great majority of authorities differentiate between a benign follicular conjunctivitis, and a 'granular trachoma," some still consider any conjunctivitis with definite follicles as a trachoma, and a few even think that folliculosis, *i. e.,* follicles on an otherwise normal conjunctiva, is "trachoma in stage of invasion."

It is true that Boldt (1904), Axenfeldt (1912), and others have given more or less detailed and clear statements of the trachoma situation as gained from their personal experiences, and as recorded in the literature, but on the whole they seem to take for granted more definite knowledge than the original records show, and hence their deductions appear too positive.

In looking up the literature we have been unable to find reports of consecutive observations on a large series of cases extending over a period of time long enough to include the supposed limits of the course of the longest trachoma cases. The lack of such published records is probably the chief reason why confusion still prevails in regard to several important points. Still a third and of course the most important reason for confusion lies in the fact that the specific cause of trachoma still remains to be definitely proved.

We began our studies nearly 4 years ago (in the spring of 1910), with these three points as the chief ones in view, *i. e.,* (1) to determine how much we really know of the condition called trachoma, (2) to study the relation of trachoma to allied conditions, and (3) to search for the specific cause of trachoma. Our general plan of work was to study minutely for a long time both from a laboratory and a clinical standpoint a large series of cases embracing trachoma and allied conditions. Some of our earlier studies have already been reported, [56], [57]—but these papers give a complete description of the work continued to date.

Critical Review of the Literature. This review which is necessarily brief, is taken up under the following heads: (1) Definition, (2) History, (3) Geographical distribution, (4) Race, Age, (5) Helping Causes, (6) Specific cause, (7) Symptoms, (8) Treatment, (9) Summary.

1. *Definition.* That our knowledge of trachoma lacks exactness is shown at the outset by the different emphasis placed upon the same points in the various attempts at definition. These attempts are made partly from a clinical standpoint, partly from that of histo-pathology, and partly from a mixed standpoint. For example, Fuchs says (1908), "Trachoma, like acute blenorrhoea, is an inflammation of the conjunctiva which originates by infection and produces an infectious purulent secretion. It is distinguished from acute blenorrhoea principally by the clinical course in which is developed an hyperplasia of the conjunctiva that forms the most characteristic symptom of trachoma. From the roughness of the conjunctiva caused by this hyperplasia the disease has received its name." In this clinical definition the points emphasized are only two, namely, an infectious purulent secretion and a chronic hyperplasia. Nothing is said about discrete follicles or, definitely, about cicatricial tissue formation. Again, Morax says "Trachoma is an infection producing nodular lesions (the granulations) situated chiefly on the tarsal conjunctiva, but may be in the conjunctival sacs and even on the cornea.

The lesions progress slowly and leave after them very often cicatrices." Here the follicles are chiefly emphasized, and it is admitted that not always is cicatricial tissue formed, and nothing is said about secretion. The Trachoma Commission of Philadelphia agrees with Morax, except they state definitely that the process progresses, if *unchecked* through its various stages of inflammation to destructive infiltration of the cornea, cicatricial contraction of eyelids and great interference with, if not total destruction of eyesight. Here for the first time pannus is mentioned and the greatest emphasis is placed upon the destructive changes as a whole. Boldt is more particular and more inclusive. He says, "Trachoma is a chronic deep and dense lymphoid infiltration of the conjunctiva (and tarsus) appearing sometimes diffusely, sometimes as circumscribed masses of cells leading to the destruction of the conjunctiva and its transformation into fibrous tissue, and showing for a time at its onset an abnormal secretion upon which its contagiousness depends." Here follicles are indicated and the hyperplasia leads to destruction of the conjunctiva, *i. e.*, to cicatricial tissue formation. Clark, Schereschewsky, McMullen and others agree essentially with Boldt. Safford sums up the definitions made chiefly from the clinical standpoint thus: "The essential clinical feature of trachoma is a hyperplastic granular destruction of the palpabral conjunctiva."

Axenfeld does not condense into a definition, he gives only descriptions of different phases of the subject.

Then come the definitions from the pathologic studies. Thus, Goldzieher says: "Trachoma is a proliferating inflammation of the vascular layer of the conjunctiva in which the perivascular cell infiltration appears in diffuse or granular form." Pascheff says: "The only true trachoma is characterized by a lymphoid tissue richly developed in germinative centers. This tissue is not specific, but is the same as granulations of the pharynx and nase-pharynx and experimental follicles." These definitions apparently contradict that of Prowazek, who says that the trachomatous process begins in the epithelium of the conjunctiva. Mutermilch tries to corelate these two ideas by the following elaborate definition. "Trachoma consists in a long series of consequent and, in an anatomic and physiologic relationship, unavoidable changes which strive for the establishment of a new and lasting equilibrium between the epithelium and the sub-epithelial tissue, and which are started by any infectious inflammatory process. It is in no sense different from other chronic pathologic processes in mucous membranes similar to the conjunctiva. The trachoma process is a complex anatomic change which strives for conjunctival cicatricial tissue."

Mutermilch also calls attention to the obvious fact that the ophthalmologist could never diagnose trachoma in the early stages if he depended upon the definitions given, since most definitions include cicatricial tissue formation.

And so the definitions go, with no point on which all are agreed. Therefore trachoma does not stand out clearly as an entity from a study of the definitions. Now for details, which give a different aspect to the definitions in several important particulars.

2. *History.* Practically all authorities are agreed that early statistics are too indefinite to be trustworthy. Without doubt several quite different diseases were included under one name. Thus Boldt says: "Egyptian ophthalmia included not only the trachoma of the present, but simple catarrh, follicular swelling, follicular conjunctivitis and blenorrhoea." And Morax says that from the original memoirs he should judge that the cases of Egyptian ophthalmia energetically attacking the armies of England, Belgium and Austria and France in the beginning of the 19th century and considered by many authors as granular trachoma, were for the most part acute contagious conjunctivitis. Fuchs and others state the same.

The association of follicular conjunctivitis with trachoma has led to still greater confusion. "Up to 1870," says Morax, "They were confounded." Then arose the dualistic and monistic theories. Later many authors insisted upon differentiating between trachoma, follicular conjunctivitis and folliculosis, and now practically all ophthalmologists agree on this point. They differ only as to their definitions of the three conditions. Boldt states that the few supporters of the "unitarian" theory (Raehlman, Mandelstamm, Lawrentzen and others), who regard follicular conjunctivitis as an attenuated form of trachoma are found almost exclusively in countries where trachoma is wide-spread, while those who believe in the "dualistic" theory are in countries more or less exempt from trachoma. Until exact definitions are settled upon—whether they be correct or not—we shall still be unable to correlate the reports of different persons.

3. *Geographic Distribution.* The same criticisms may be made in regard to statistics on geographic distribution. Until we are at one in our definitions we cannot be sure of our limits of distribution. But the greatest difficulty now is, as Boldt points out, that "owing to the slow and insidious course of many of the cases with a vast number never coming under medical treatment it would require exhaustive investigations of the whole population of a district before reliable statistics could be drawn up." Until this can be done we have to rely upon relative figures and general statements which are often quite misleading.

All countries reported as hotbeds of trachoma *e. g.,* Egypt, Arabia Russia, Poland, Finland, Armenia, Ireland, etc.), show also many infections with the Koch-Weeks bacillus and the gonococcus, as well as with many other germs pathogenic for the eye. Hence in these areas, it is difficult to determine the specific effects of each infection. Fuchs calls attention to the fact that there are no good statistics of the relative frequency of these eye diseases. And we shall not have them, I repeat, until many consecutive cases have been under recorded observations made from minute cultural and clinical study throughout the entire course of each and all of these diseases.

This minute study has been done least of all in those districts where trachoma is reported as endemic. In this country, for example, in the mountians of Kentucky and on the Indian reservations, trachoma is said to be prevalent and increasing, so occasionally one investigator or a group

is sent to these areas for a few weeks or months. Result—a report of the number of existing cases diagnosed as trachoma! And we are no nearer a knowledge of the real nature of "trachoma" than we were before. If this work could be planned so that comprehensive records started by one group of investigators could be carried on by another over the required number of years we would be in a position to settle the boundaries of at least some of the questions relating to these conjunctival infections, such as insidiousness, intermittency, chronicity, contagiousness, etc.

The Indian reservations are ideal places for keeping such records, since they are under direct government control and the individual case can be easily followed. Especially favorable would be the Indian boarding schools in which Schereschewsky reports such a high percentage of "trachoma" *e. g.*, 90 per cent. in one school in Oklahoma.

Of course until we can show a specific cause we cannot be sure of any statistics for a disease reported to have such manifold variations. But if we have recorded descriptions of the whole course of a large number of consecutive cases we may be able more readily to detach one group of cases from another and to place each group geographically. At present the maps prepared by certain authors are necessarily inaccurate.

4. *Race and Age.* In general, authorities are agreed that no race is exempt. Reports have given the negro less susceptibility than the other races but this may be because fewer, as a whole, have been examined. McMullen says that numbers of well-marked cases of the negro from the West Indies are annually detected at Ellis Island.

All are now agreed that no age is exempt, though this point also has been much disputed.

5. *Helping Causes.* There is no dissenting voice from the conclusion that trachoma is a disease above others that is fostered by ignorance and filth. Hence it is found to be first a disease of families among the poor. "The family is the incubator." Cases appearing among the well-to-do are only apparent exceptions. Treacher Collins[3] cites an instance in point: "In South Australia . . . the disease . . . is met with in well-fed, strong, healthy farmers and their families, who . . . reside in roomy, well-ventilated houses. South Australia is, however, a riverless country. . . . Each individual farmer has to arrange receptacles for the storage of surface flood water, and the greatest economy in the use of this conserved water has to be exercised to make it last over the rainless summer months. Where scarcity of water exists, regular and proper ablutions amongst the inhabitants are sure to be neglected and the possibilities of the transference of contagious diseases of the eye greatly increased. . . . The other accompaniments of a hot, dry district which predispose the conjunctiva to infection, and which aid in the introduction of infective matter into it are also present, *viz.*—frequent dust storms and abundance of flies."

Morax says: "Cleanly Arabs have little trachoma, dirty ones have much." Very recently both McMullen and Stucky have given detailed

accounts of the unhygienic habits of the Kentucky mountains cases, and Schereschewsky of those of the Indians.

That the disease is of a milder type to-day ophthalmologists say is due to the more effective carrying out of better hygienic measures everywhere. When we consider how far this still is from the ideal we need not wonder that chronic eye conditions still prevail.

Anything that lowers general vitality, such as tuberculosis, may, of course, aid any disease. Anything that lowers local vitality appears to be of greater importance in these eye diseases. As early as 1861 Weiss considered that trachoma could occur as a sequel of old conjunctival inflammations. And Boldt says: "All chronic irritating conditions of the eyes, especially, obstinate conjunctivitis augment the tendency to trachoma. . . . Individual predisposition is increased by external sources of irritation. Among these dust and smoke are of prime importance, for by long continued action they set up chronic conjunctival irritation. Further, heat, moisture, wind, want of sleep, over-crowding and bad ventilation must be mentioned."

Since dirty habits and lowered general and local health also help produce conjunctivitis from whatever cause, a study of the helping causes of trachoma does not aid in making the disease an entity.

6. *Specific Cause.* Nearly all authorities agree that trachoma is an infectious disease spread by contact, but a number of investigators doubt that a single specific organism is the cause. The conjunctival sacs are such favorable localities for the growth of several different varieties of micro-organisms—especially when invaded by one variety, that the flora obtained from any one examination may be quite large. For this reason it has been easy to admit the theory of multiple causes. Thus, among others, Cazalis, who made an extensive study of the etiology of trachoma in 1896, came to the conclusion that bacteria of the various forms of conjunctivitis can, under certain condition, aided by the patient's predeposition produce trachoma. Later Mutermilch, Pascheff, and others, state that trachoma in no respects differs from other chronic inflammatory processes occurring in mucous membranes whose structure is related to that of the conjunctiva, such as chronic urethritis, atrophic rhinitis, chronic appendicitis, etc. They conclude that trachoma belongs to the class of typical chronic inflammations of the mucosa, produced by various causes.

For this reason, too, several other research workers have made claims in favor of one out of a number of known microorganisms as the specific cause of trachoma. Thus the gonococcus, the streptococcus, Koch-Weeks bacillus, the influenza bacillus (Müller's bacillus?), and various other micrococci and bacilli, as well as yeasts, moulds, protozoa—each has had its day, only to be dropped in favor of a new organism when a new investigator happened to find a few cases in which one or the other of these microorganisms seemed to predominate. Some of these hypotheses have lingered longer than others. Some, we have with us still; for instance, the gonococcus hypothesis (Alt, Herzog); though Morax says (1906), "It is not necessary to discuss the relation of gonorrhoea to trachoma. Granulations occurring as the result of gonococcus infection

are of short duration and do not cicatrize." Mutermilch, on the other hand, calls attention to the prevalence of gonococcus infection in the Egyptian trachoma cases and comes to the conclusion that the gonococcus must have some part in the process. Every once in a while new claims are brought forward in favor of the gonococcus. The latest, by Herzog, since the discovery of the "trachoma or Prowazek inclusions," is that the gonococcus may become involuted into these granular inclusions.

Claims for ultra microscopic forms (Raehlmann, Bertarelli), have also been brought forward, but Pfeiffer and Kuhnt were unable to get positive results from the use of filtrates into human eyes.

And now, in our present report, we are presenting strong evidence in favor of the influence of the group of the hemoglobinophilic bacilli in chronic conjunctivitis; at least evidence that these bacilli may produce a hyperplastic inflammation which may result in cicatricial tissue formation. So we shall give more details of the bibliography of this group as related to the pathology of the conjunctiva.

The Koch-Weeks bacillus which is placed in this group, though most authors say it can grow without the presence of hemoglobin (this question of growth will be taken up in the original work), has been frequently reported as occurring in trachomatous eyes from the time of Koch (1881). Thus Collins (1909), says, "In many cases of trachoma in this country (England) now the Koch-Weeks bacillus is found in the muco-purulent discharge from the conjunctiva, which occurs during an acute exacerbation of symptoms. The ophthalmia introduced by the troups from Egypt was most likely, then not one disease, but a mixed type." Müller says, "In the majority of adults with trachoma the Koch-Weeks bacillus was found." Mutermilch goes farther in declaring, "Often repeated infection with the same microorganism, *e. g.*, bacillus of Koch-Weeks, produces a series of exacerbations on an already inflamed conjunctiva and finally produces the picture of trachoma." Markus states that the Koch-Weeks bacillus is the cause of "Schwellungskatarrh" (our papillary conjunctivitis).

Many of the bacteriological examinations have been made by smears alone, hence the identification of the bacteria was made chiefly from morphology. This means, as we have shown, that many may have been missed. Moreover, successive cultures from the beginning to the end of the disease, have seldom been made, neither have hemoglobin-containing media often been employed. Indeed, seldom, if ever, has minute search been made on a large scale for either the hemoglobinophilic group of organisms or for the gonococcus in these chronic eye infections. Earlier reports were taken for granted as representing the whole truth. When we realize that the specific organism may be scanty in the later stages of a disease or may be difficult to isolate then because more deeply seated or because of over-growth of secondary organisms we know that we cannot infer much from negative reports based on few examinations. Then, too, and this is a most important point, only a comparatively few investigators have used blood media, and none of them,

as far as we know, except ourselves, has used an enriching blood medium. Hence, doubtless, we are far from determining the prevalence of hemoglobinophilic bacilli in these eye infections.

Müller who isolated hemoglobiniphilic bacilli from the largest series of trachoma cases reported, recognized the importance of more than one examination. He considered his comparatively small percentage of positive cultures due chiefly to the fact that he could make *only one examination* in many of his cases. He had positive results chiefly in his "acute trachoma' cases. Muller thought at first that his bacillus was the cause of trachoma, but now, without positive proof, most authors have decided that it has nothing specifically to do with trachoma (zur Nedden, Morax). Thus Morax says, "It is not difficult to show that this bacillus has nothing to do with the production of granulations." But did he or any one else show the effect of repeated infections with it on a case of simple follicular conjunctivitis, or on an otherwise susceptible conjunctiva, in producing hyperplasia and finally fibrous tissue? That it is thought to be identical with the influenza bacillus capable of invading secondarily a trachomatous conjunctiva and producing a superimposed influenzal conjunctivitis does not help much to clear up its relation to "trachoma."

In fact, practically no detailed account has been made in the published reports of the number and minute character of attacks of "intercurrent" diseases throughout the course of trachoma, and of their relation to the "exacerbations of trachoma." Only general statements have been made of numerous successive attacks of conjunctivitis, both leading up to trachoma and occurring throughout its course. Thus Greef says that the chief cause of conjunctivitis in Egypt is not trachoma but acute catarrh produced by the Koch-Weeks bacillus, and chronic catarrh produced by the Morax-Axenfeld bacillus. Dwyer and Meyerhof point out the difficulty of securing fresh cases of trachoma in any but infants who are apt to show at the same time both gonococcus and Koch-Weeks bacillus infection. Though Axenfeld and others recognize the fact that the Koch-Weeks bacillus and the gonococcus occur frequently in eyes in Egypt and elsewhere, producing acute attacks on "trachoma," they do not show in how many of these eyes these bacilli continue to exist and to produce a subacute or chronic inflammation. Neither do they consider the result of a reinfection with new strains of either of these organisms in eyes already made sensative to them. All they say is that they *think* that the present "mild type of trachoma" is due to the less frequent occurrence of the Koch-Weeks bacillus and the gonococcus.

Blumberg, in adopting the theory of multiple causes states that recurrent attacks of conjunctivitis diminish the resistance of the reticular connective tissue of the conjunctiva and cause a continual state of irritation which finally produce trachomateous changes. Boldt in quoting Blumberg, goes on to say, "If we consider predisposition to consist in overgrowth of adenoid tissue, as Peters does, Blumberg's theory becomes very plausible, and we can readily understand how repeated

chronic inflammatory conditions of the eye produce sponginess, atony and a tendency to proliferation in the adenoid tissue."

We can clearly see from the above brief sketch that the question as to the ultimate influence of these bacteria on the eye is far from settled. We have demonstrated [55] the continued presence of hemoglobinophilic bacilli in cases showing successively acute, subacute and chronic inflammation of the conjunctiva and their increase in number during acute exacerbations. The following reports take up this question more fully.

In the meantime came the work of the *inclusionists*. The bibliography of this work has been gone over so fully both by this laboratory[17] and by other sources that the chief points may only be touched upon here.

Halberstaedter and Prowazek (1907) described certain bodies in the conjunctival epithelial cells of cases diagnosed by them as trachoma. Greef, Claussen and Frosch independently described similar bodies. Unfortunately they did not go into details as to the course of their cases so we do not know just where to classify these in a disease of such protean descriptions as trachoma. Halberstaedter and Prowazek simply state that the inclusions were found more frequently in fresh cases. The same indefiniteness as to clinical course is shown by most of the authors following them. We can only gather that the bodies were found in largest numbers in "acute trachoma" or in "acute exacerbations of old trachoma."

Then came others who said that similar inclusions are found in acute conjunctivitis cases of the new-born, also in vaginitis, urethritis and even in the normal conjunctiva.

As is usual after the report of a new finding, confusion has arisen as to how many manifestations may be considered a part of the described bodies.

From the accumulation of evidence gained from the vast amount of work done by others on this subject as well as from our own work extending over four years we have come to the conclusion that there are inclusions in the conjunctival epithelial cells of certain cases of conjunctivitis, answering in the main to the descriptions of Halberstaedter and Prowazek and others, which are foreign invaders. We further believe that these foreign invaders may enter the epithilial cells of other mucous membranes of the body but that they could not enter the normal cell in appreciable numbers without producing an inflammatory reaction. Hence we believe that the bodies found in the normal conjunctiva (McKee, Addario), are simply incidental cell changes. Indeed the descriptions and plates of the above named authors show this.

We have already called attention [55], [56] to the similarity of the "typical inclusions" found in cases of papillary conjunctivitis ("acute trachoma") to nests of the hemoglobinophilic bacilli. This work will be taken up more fully in the body of the report. This idea differs from that of Prowazek in regard to their nature. He has created for them a group which he calls the Chlamydozoa—or mantle animals—and places it between the protozoa and the bacteria. Prowazek seems not to be aware of the limits of morphologic change in bacteria.

Apparently we have no definite help in making trachoma an entity from the studies of its etiology.

Nor does the study of the reports on the *contagiousness* of trachoma help us. While all but a very few authors agree that trachoma is contagious they differ as to the degree and stage of contagion. Again most statements are general and the few specific statements are not backed up by enough clinical evidence to draw trustworthy conclusions. Thus Boldt says, "Experience teaches that infection does not readily occur but usually only after prolonged contact with the patient and common use of beds and washing utensils. Then one person after another is slowly infected, therefore we can only claim a restricted and conditional contagiousness for trachoma, not underestimating the significance of individual predisposition." So, too, Axenfeld, Morax, Fuchs, Doyne, Clark and most others believe that trachoma must be placed among the less contagious, or slowly contagious diseases. On the other hand a few authors say that it is highly contagious (e.g. Trachoma Commission of Philadelphia, etc.)

All agree that "the danger of contagion is in direct proportion to the amount of secretion," for, they say, "the specific secretion may be enhanced by a superimposed infection." But the possibility that the "superimposed infection" may produce a hyperplastic or an atrophic inflammation seems to be lost sight of except by the few. It is true Morax comes near it when he calls attention to "the light cases—the subacute and chronic cases of Koch-Weeks bacillus infection" which "are considered non-important but which may give rise to more serious ones."

In these subacute and chronic cases, as we have showed, the secretion may be so slight that it may be easily over-looked, and even when found, the stained spreads from it may show no recognizable bacteria. Cultures, however, may show that bacteria are present. These bacilli with their possible change in virulence may insidiously attack another eye which may be in a condition to allow a chronic inflammation instead of an acute one.

We know too little either about the condition producing virulence in certain groups of bacteria, or about the conditions influencing susceptibilty to their pathogenic action summarily to exclude them from a list of causes producing any degree of proliferative and fibrous changes in tissues.

7. *Symptoms.* Once more, most statements are naively limited or indefinite. The disease is divided into types (severe and mild, papillary and granular, acute and chronic, etc.) and these types into stages (such as, development, regression, cicatrization, etc.). Different terms are used for apparently the same type and stage by different authors which helps make confusion worse confounded. The trouble is that the course claimed for this disease is so protean in its variation and, as Collins points out, is "usually of such exceedingly long duration that few observers have had the opportunity of watching cases from commencement to finish," and therefore of getting at a clinical entity if it exists. These confused ideas may be more easily seen by a consideration of the claimed symptoms.

Onset. Some authors say the disease always begins acutely with secreting conjunctivitis (Collins, Reese, Schereschewsky, etc.). Others are just as emphatic in stating that it always begins insidiously with follicle formation and without visible secretion (Weeks, Trachoma Commission of Philadelphia, Stiel, McMullen, etc.); while a small majority take the middle course and consider that though there are undoubtedly acute secreting cases they are now few (Boldt, Morax, Fuchs, May, Harrison, etc.). But since all authors are agreed that "one rarely sees a beginning follicular or granular trachoma and follows it to its terminal cicatrization," it can be seen that the character of onset as a whole in this disease must still remain a question.

Secretion. While there is much doubt as to how often if ever trachoma begins with a secreting conjunctivitis, there is very little difference of opinion as to the presence of a "specific secretion from time to time during the course." But how to differentiate between a "specific secretion" and that caused by a superimposed infection seems difficult. In fact, few attempt it and these most unsatisfactorily. Thus Morax says: "At a certain degree [?] of development there is always produced a slight muco-purulent secretion. When the secretion is more abundant [?], when the lids stick much [?] on waking, it is nearly always the result of a superimposed infection [sic!]." Farther on Morax says, "Probably all cases characterized by a purulent or mucco-purulent secretion are mixed infections." Of course the majority of ophthalmologists say they depend upon the microscope for differential diagnosis; but this generally means one examination by smears alone, and as we have shown above, this is insufficient to rule out a number of bacteria. Stiel says: "In pure trachoma, secretion of the conjunctiva is not necessary; on the contrary secretion always means a mixed infection." Considering the prevalence of bacteria causing all grades of secreting conjunctivitis, the reported susceptibility of trachomatous eyes to mixed infections and the prevalence of all conditions favoring such infections it is highly improbable that anyone could demonstrate the course of any case of "pure trachoma" going on to cicatrization *without secretion* appearing at times throughout its course. The statements, therefore, "that secretion is not necessary" and "that only a slight secretion is specific" are matters of opinion only.

Follicles. An immense amount of study has been carried on concerning the characteristics of follicles in trachoma, with the result that no definite clinical or microscopic differences between the follicles of "trachoma" and of other conditions have been demonstrated. While the majority of authors believe that "there is essentially only one form of trachoma, that is, "granular trachoma," they cannot agree in differentiating trachomatous granulations from follicles found in any follicular conjunctivitis except by stating that the former are "follicles which lead to a permanent injury of the eye" which opinion doesn't help in early diagnosis.

Cicatricial Tissue Formation. Here we have a difference of opinion again. Some authors say: "Always some cicatrical tissue is formed

however light the case and whatever treatment (McMullen, MacCallan, etc.). Others think with Boldt, "We no longer consider connective tissue formation and scarring to be an inevitable result as Saemisch once did, because it is frequently possible by early and appropriate treatment to stop the disease before the appearance of the cicatricial stage." Then it has been shown that certain known infections may produce cicatricial tissue, e. g., Morax-Axenfeld bacillus and gonococcus in the eye, influenza bacillus in the lungs, etc.

Pannus. Here, tho there may be a question as to the limits of "specific pannus," practically all are agreed (even those who put pannus under complications and sequellae (sic. !), that such a pannus exists but that it seems to have no relationship to the intensity of the infection as evidenced by follicle formation since it may occur at any time throughout the course; however, it is supposed to be more serious when it appears late in the disease, *i. e.,* after the establishment of cicatrical tissue.

Diagnosis. Considering all of the above conflicting statements in regard to symptoms, there is little wonder that when it comes to a question of early diagnosis, no one seems to be able to give any aid. A "good diagnostician" may claim that he can make an early diagnosis, and he proceeds to say: "This is a case of trachoma." He may even give his reasons. But how many of these early cases are followed up to determine whether, according to any of the various opinions, the diagnostician is right or wrong? It is not surprising that all authorities are agreed that "in the beginning the diagnosis must be held in suspense." On *one examination* a certain diagnosis can only be made in *old, typical* cases. This is simply saying that eyes showing such changes are trachoma and leaving out of consideration the question as to how many different agents may cause pannus and conjunctival cicatricial tissue.

Prognoses, it goes without saying, are also various. Some say there may be spontaneous cures, some even claim that there are abortive cases which heal without scars; others assert that unless treatment be instituted in a very early stage trachoma is never cured; some say that with early treatment there may be complete recovery without scarring; others say that however early the treatment, there is always scarring, etc. Again we may take our choice.

In Treatment we have another rather wide limit of choice. As far as *prophylactic treatment* is concerned all are agreed as to its importance but few have carried it into full practice. Most of the little that has been done has been directed against follicles and not against acute conjunctivitis or the lighter subacute cases where no follicles may be present in the beginning. Until we so realize the importance of the relationship of acute conjunctivitis to trachoma that we shall employ aids on every side, such as are shown in our report on prophylaxis (pp. 182-213) we may expect tardy results in ridding us of "trachoma."

The great majority of the treatises on treatment have to do with more or less advanced cases, *i. e.,* with *curative treatment.* "Trachoma" is said by most authorities to be comparatively easily cured, after at least several months of treatment under the following conditions: (1) When

there are no complications; (2) when in children, or (3) when cases come early enough. All of which statements might be applied to any form of chronic conjunctivitis.

The principle of treatment in the advanced cases is that used in any chronic sluggish inflammation, *i. e.*, to stimulate. Several authors emphasize the importance of change of stimulants. On the other hand Boldt says that early slight cases or chronic torpid ones often do better under simple hygienic treatment.

Opinions differ now so widely in regard to the merits and applicability of operative treatment, *i. e.*, expression, grattage, excision, that a study of them gives us little help in determining the entity of trachoma.

Summary

In summing up the mass of writing on trachoma we find that there is no widespread agreement as to the claimed essential points. The chief lack seems to be, as I stated in the beginning, that there has been no critical following up of a large series of cases over a long period of time, with studies directed especially to the following particulars:

1. Written records kept of each case from the beginning to the end of the disease.

2. Successive cultures as well as microscopic examinations made of each case using enriching media to detect those organisms that grow with difficulty, combined with inhibiting substances for organisms which might overgrow more important ones.

It is hoped that the following reports will help show that this lack is being supplied.

II.—ORIGINAL WORK

A. Clinical Studies

1. DIAGNOSIS AND COURSE OF FOUR THOUSAND CASES OF CONJUNCTIVAL AFFECTIONS, INCLUDING TRACHOMA AND ALLIED CONDITIONS

By Herbert W. Wootton and Anna I. Von Sholly, Assisted by Caroline R. Gurley, Percy Crane, Alice Kurtz, and Ella Lipsky

To the medical profession it seems hardly necessary to state that the diagnosis of trachoma, except in the cicatricial form, is an exceedingly difficult matter, the more so in view of the fact that the existence of such a pathological entity has recently been doubted. Is there or is there not a specific chronic inflammatory affection of the conjunctiva frequently accompanied by a characteristic involvement of the cornea, which begins with thickening of the adenoid layer and the localized accumulation of small round cells of various kinds, which are gradually transformed into granulations and cicatricial tissue? This question has been answered in the affrmative by most of the better known writers upon ophthalmology and this description of the disease has been regarded as classic. Recent investigations, while not conclusive, have tended to throw doubts

upon this time-honored theory and to suggest that trachoma, far from being a disease *sui generis,* consists rather of a series of infections possibly of similar, perhaps of dissimilar, character, which, at certain stages, may be recovered from under treatment or spontaneously, but which, after a certain period, results in cicatricial formation. This theory has a certain amount of fascination. It tends to explain why, although trachoma is on all sides believed to be contagious, no specific organism of the disease has been discovered despite the most painstaking search. It also coincides with the known fact that trachoma is a disease of no special race or country, but is essentially a manifestation of uncleanliness and unsanitary surroundings. A person of ordinarily cleanly habits who contracts, let us say, an acute catarrhal conjunctivitis, and pays proper regard to cleanliness and treatment, is speedily cured and is not liable to immediate reinfection from his surroundings. The case is different, however, with the poor living for example, in hovels in Eastern Prussia. Here an attack of acute catarrhal conjunctivitis would not receive proper treatment. It would assume a tendency to pass into a subacute or chronic stage, would infect other members of the family, and would, in turn, be reinfected by them. Thus the irritation caused by the original infection would tend to become more or less continuous and, unless checked by treatment and a change in hygienic and sanitary conditions, would ultimately result in the production of cicatricial tissue. This theory is as difficult to combat as it is to prove for the reason that, until some one has been able to follow closely a sufficient number of cases of conjunctival affection from their incipiency to their termination in cicatrization, and at the same time to rule out other infections which might produce fibrous tissue, a study which, so far as we know, has never been done, we shall not be able to state that trachoma as a pathological entity exists.

This is exactly what the studies reported in this series of papers are attempting to accomplish.

At the beginning of this work we established a special clinic in connection with the Research Laboratory in order to allow clinical and laboratory observations to proceed closely together.

Here we developed the folder system of records, the value of which in this kind of a study cannot be too highly praised. The outer folder contains the previous history of the case, the family history, notes of home visits and the original and supplementary diagnoses of the case with the results of miscroscopic and cultural studies; while the inner cards which may be added as needed contain a minute description of the different phases in the course of the case. A copy of the outer folder (front and back) of one of these case records is given here.

[FRONT OF FOLDER]

Name	Place of Birth	Age	Public School	Date Admission	Case Number
Celia. K	W.S.	9yrs.	22	5/27/11	1436

Address

X

DIAGNOSIS

1. Original - (a) Clinical 5/27/11. Follicular Conjunctivitis (mild).
(b) Microscopic + "trachoma inclusions," + hemoglobinophilic bacilli.

2. Complications —

3. Supplemental Diagnosis 7/27/11. Papillary "trachoma" (later called papillary conjunctivitis).

HISTORY

4. When did eye trouble begin? Dec. 1910.

5. Subjective Symptoms (a) Onset Ocular injection; lids stuck in mornings.
(b) Now None

6. TREATMENT—(a) When? Dec. 1910 (b) Where? Gouverneur Clinic (c) Nature of Treatment

7. OPERATIONS (a) How many? — (b) Where Performed? — (c) When? —

8. DISCHARGED—(a) Date 1/28/13 (b) Condition Normal

FOLD ON THIS LINE

10. RELATIVES

X	RELATIONSHIP	NAME	AGE	PLACE OF BIRTH	EMPLOYMENT OR SCHOOL	TIME IN U. S.	DATE OF EXAM.	EYE CONDITION	CASE NUMBER
	FATHER			Austria		12 yrs.			
	MOTHER			"		26 "	4/3/11	O.K. 4/7/11 O.K.	
	Sisters	Sadie	10	W.S.	P.S. 98				1165
		Rose	8	" "	" "		4/1/11	O.K. 4/9/11 O.K. 7/24/11 O.K. 10/5/11 O.K.	
		Lillie	6	" "	" 88		"	" " 4/5/11 " " " " " 3/28/12 "	
		Esther	15 Mo.	" "			"	" " 4/7/11 " " " " "	
	Brother	Peter	3				"	" " " " " "	
	Grandfather						4/1/11	O.K.	

11. No. of Boarders 0 (a) Nationality Austrian (b) How long in U. S.?

12. HYGIENIC SURROUNDINGS (a) No. of Rooms 3 (b) Other Occupants of Sleeping Room Adult 0 Children 1 (c) Does Patient Sleep in Separate Bed? ~~Yes~~ No

(d) If not, with whom? Child ✓ Adult (e) Separate Towels? ~~Yes~~ No (f) Separate Pillow Cases? ~~Yes~~ No (g) CLEANLINESS ~~Yes~~ No

RESEARCH LABORATORY—DEPARTMENT OF HEALTH OF THE CITY OF NEW YORK.
Form No. 136N

Inspector

467-12 (Bt.) 5000

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[BACK OF FOLDER]

INSTRUCTIONS

When further comment on point is desired put "X" in column marked "X" against the point, and enter number in column marked "X" on Supplementary page.
Write first—date of interview, name of person interviewed (giving initials) and address.
Paragraph freely.

DO NOT WRITE IN THIS SPACE	X	SUPPLEMENTARY NOTES
Home Visits. 4/1/11		See front of sheet.
5/27/11		December last, eyes red and stuck in mornings; went with sister to clinic, not told nature of trouble; mother says no trouble now; never was kept out of school; mother says redness lasted only a day or so.
6/5/11		Celia. Tarsal conjunct. practically normal except for few fine follis. lymphatic dilatations lower lids. Lillie. O.K
6/7/11.		Sadie. Tarsal conjunct. normal color vessels seen, moderate number translucent follis. on Tarsus and in canthi. Lymphatic dilatations lower lids. Follie. Conjunct.
7/26/11		Celia. Conjunct. upper lids very much thickened: papillary condition; vessels not seen; mucopurulent secr., lymph. dilatations of lowers. Looks like "Trachoma." (Goes to Gouverneur Clinic three times a week). Sadie. Tarsal conjunct. some what thickened, with numerous follis.; vessels obscured; slight secretion, ptosis right eye. Mother very shiftless and indifferent.
9/25/11		Celia. At Convalescent Home at Wilton, Conn.
10/10/11		" " " " " " " ; has gained 4 lbs.
2/24/12		Celia. Eyes still paste in morning.
3/25/12		Mother preparing for holidays. Walls just painted, but everything else extremely untidy and dirty. Mother seems rather suspicious or indifferent, certainly not interested in regard to instructions; says too busy to attend demonstrations now. Eyes O.K. (but condition favorable for re-infection). Celia and Sadie now go regularly to Housekeeping Classes at 226 Henry Street.

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TABLE I

Chief Clinical Points Noted in Course of 4,261 Cases Under Observation

Original Diagnoses	Later Diagnoses	Number	Follicles	Pannus	Cicatricial Tissue	Operations		Present Condition				Number Lost Sight of
						Before Seen	After Seen	Unimproved	Improved	Cured		
										Number	Average Time	
Old Trachoma	Old Trachoma	59	48	32	59	25*	3	10	36	4	3 yrs.	9
Acute Hypertrophic or Papillary Trachoma	Papillary Conjunctivitis	181	162	8	58†	64	6	8	19	136	9 mos.	18
Doubtful Trachoma	Papillary Conjunctivitis	161	161	3	49†	58	9	6	15	123	10 mos.	17
	Follicular Conjunctivitis	20	20	0	5†	7	0	0	0	19	1 yr.	1
Follicular Conjunctivitis	Papillary Conjunctivitis	16	16	1	3†	4	2	2	6	7	1 yr.	1
	Follicular Conjunctivitis	665	665	0	45†	51	3	0	69	596	1 yr.	(?)‡
Acute Catarrhal Conjunctivitis	Papillary Conjunctivitis	20	8	2	2†	3	1	1	4	15	6 mos.	0
	Acute Catarrhal Conjunctivitis	700	105§	0	0	0	0	0	0	700	6 wks.	(?)‡
Folliculosis	Papillary Conjunctivitis	5	5	2	2	3	0	0	2	3	6 mos.	0
	Folliculosis	2120	2120	0	0	0	0	0	0	2120‖	1 yr.‖	(?)‡
Miscellaneous	Blepharitis Phlyctenular Conjunctivitis, etc.	300	190	0	0	0	0	0	0	294	3 mos.	(?)‡

* It was often impossible to get clear histories from these old cases. Probably all have had at least one operation for expression.

† Only operative scars presumably.

‡ These cases were not all examined at the present time. The great majority, at least, are still in our schools and are sent to our clinics from time to time for examination and immediately, if inflammatory symptoms supervene.

§ Mild folliculosis, chiefly after acute process had subsided.

‖ With the exception of mild folliculosis in a comparatively small number of cases.

During the first half of our work all of the diagnoses and descriptions were made by the one in charge of this part of the work (Wootton), but latterly other ophthalmologists have assisted, and finally when the ophthalmia school and the school clinics were started, this part of the work was definitely in charge of another of the group (Von Sholly), doubtful cases only had been referred to the director who visited the clinics from time to time. It can be readily seen how essential are full written descriptions of each case.

Even with these descriptions it has at times been difficult for us to come to an understanding as to each one's meaning of hypertrophy, thickening, cicatricial tissue, characteristic follicles, kinds and amount of secretion, etc. For example, we must ask the question, is the term hypertrophy used to mean true hyperplasia, that is, proliferative inflammation, with the formation of cellular connective tissue; or may it be also used to mean only a more or less temporary infiltration of lymph cells. Clinically, of course, it may be impossible to decide this question until late in the course. Again, we found that a small amount of secretion gathered in the superior cul de sac was not recorded by some examiners; though the fact of its presence is of importance in accounting for the finding of hemoglobinophilic bacilli in cultures from those chronic cases reported as having no visible secretion, in accounting, too, for a focus of infection.

In this way more or less accurate and continuous observations, extending over a period of about four years, have been made of some four thousand cases of conjunctival affections occurring for the most part in the public school children of New York City. These observations tend to confirm the statement of Fuchs and others that, in children, trachoma as it is described in its entirety is a rare disease. The affections of the conjunctiva most common in this series of cases which have, from time to time, been regarded as trachoma by various authors, may be described under four headings.

First—Folliculosis or pronounced type of follicular formation without evidences of inflammation. The writers would describe follicles clinically in this group of cases as situated either upon the surface of the conjunctiva or in its substance. They may be situated on any part of the conjunctiva of both lids, but everywhere arise from a normal base. The underlying conjunctiva is not thickened and its blood vessels are perfectly distinct. In the upper, and more especially in the lower, fold of transition, hemispherical or ellipsoidal, yellowish translucent bodies are also discernible. These are less firm than the follicles and are simply an accompaniment of the follicular affection and may or may not be present. They are also sometimes observed upon the ocular conjunctiva, and are said to be simply dilated lymphatics. There is no secretion and the eyes are not injected. There are no clinical symptoms, even when the follicles are present in a marked degree. This form of conjunctival affection never terminates in cicatrization and is never accompanied by corneal involvement.

That these cases, uncomplicated, are harmless is shown plainly by the course of our series of cases given in Table I. Only three of these cases were operated upon, and only two showed, about one year after first seen, a conjunctivitis during which pannus developed. All of the others—over two thousand cases—have now either normal conjunctivas or only a few small follicles.

Second.—Follicular Conjunctivitis. In this variety follicles are present as before and the "lymphatic dilatations" may or may not coexist. The mucosa, however, is injected, and secretion is present, for the most part watery, and the eyes appear suffused. Mucous or muco-purulent secretion may also be present from time to time. There is no thickening of the conjunctiva surrounding or underlying the follicles. These cases would, according to the theory above described which denies the existence of trachoma per se, be capable, unless cut short by treatment, of becoming hyperplastic and terminating in cicatrization. One occasionally sees in children, though rarely, cases presenting at the same time the follicular formations, secretion and pannus. This fact may be held to support either the theory in question or the theory that there is such a pathological entity as trachoma, according to the point of view.

In most of those cases which, according to our observations, have presented conjunctival thickening (hyperplasia), as well as follicular formation, the follicles have been larger than in the cases we are prone to call follicular conjunctivitis. In some adult cases which one of us (Wootton) has seen, the presence of follicular formation deep in the conjunctiva itself has been evidenced by the occurrence here and there of small yellowish infiltrates. Such cases have been called trachoma, since pannus has been seen to occur in several of them. No case clearly of this type has been seen in the series of school children here studied. Among the 671 cases diagnosed when first seen as follicular conjunctivitis, 16 developed a subacute papillary condition later, which cleared up without showing pannus.

Third.—Papillary Conjunctivitis. The characteristic feature is thickening of the conjunctiva, presumably through infiltration with lymph cells and wandering connective tissue cells. Follicles may or may not be visible, and when present do not form an essential feature of the disease. The conjunctiva is thickened and red; its blood vessels are invisible or obscured; muco-purulent secretion in varying degree is always present. In these cases the so-called trachoma inclusions or bodies of Halberstaedter and Prowazek are so frequently present that they may be regarded as a constant feature. For this reason, in the early part of the studies upon which this article is founded these cases were regarded as trachoma.

In following the course of these cases, we found that practically all of them recovered after presenting a subacute or chronic condition for a varying number of months, without *having developed either pannus* or *cicatrization.* All of these cases were treated, most of them from the beginning, both at home and in the clinic, in our usual way (pp. 182-213), so we cannot be sure what course all of them might have followed without

such treatment. All we can say is that our observations lead us to support the suggestion of Cohen and Noguchi that "trachoma inclusions" are micro-organisms which may produce a conjunctival affection having nothing to do with classic trachoma. From the work of Williams and others of our group (p. 222), it would seem that the "trachoma inclusions" in this type of cases are nests of hemoglobinophilic bacilli growing in the epithelial cells of the conjunctiva. This latter hypothesis supports the observations of Markus and others who have stated that members of the group of hemophilic bacilli are the cause of papillary conjunctivitis (Schwellungskatarrh).

The fourth type of cases studied in this series may be classed as *Trachoma.* They are the cases which present the well-known characteristics of cicatricial degeneration or corneal involvement or both. In our table we have classed all of those that showed these characteristics when first examined by us as "old trachoma," excepting 8 cases of papillary conjunctivitis with pannus which gave a history of a moderately short course. The few cases of cicatricial tissue formation which developed during our four-year period of observation were post operative cases and the "scar tissue" apparently was a result of operation. These and the pannus cases are indicated in the "course" columns of Table I. From the study of the group of cases classed as "old trachoma" in our table we can infer little, since we were not able to study them from the beginning. But from those proceeding to cicatricial tissue formation (without operation) and pannus while under our observation we may hope to gain more light.

In favor of the classical theory that trachoma is a disease per se, we must not lose sight of the peculiar character of the corneal involvement which so often occurs when the conjunctiva shows cicatricial changes. This keratitis, designated by the special name pannus, consists in an infiltration of small cells between the superficial epithelium and Bowman's membrane, which proceeds from above and is accompanied by vascularization from the adjacent conjunctiva. It may gradually invade and destroy Bowman's membrane. In our series of cases we have found a condition answering to this description in 46 cases. In 16 there is an acute history, and as yet no cicatricial formation has been observed in any of these cases. Eight of these 16 cases developed the pannus while under our observation, while the other eight showed beginning pannus on first visit and gave a history of an acute process. The other 32 cases were all old cases when first seen by us. The microscopic findings in these cases are given on page 239.

Let us now turn for a moment to the consideration of the corresponding conjunctival affections of adults. The pure follicular type (folliculosis) is exceedingly rare, but when it does occur is no more liable to disagreeable sequellae than in children. The second type, follicular conjunctivitis in a pronounced form without thickening of the conjunctiva, is, we should say, practically unknown in persons over sixteen years of age. Papillary conjunctivitis is found in adults and presents the same characteristics as in children.

The terminal stage of trachoma, represented by cicatrization of the

conjunctiva and pannus, is much more common in adults than in children. Most of the cases in New York City occur among the foreign population, probably as the result of infection in foreign lands. These cases are decidedly less frequent in our clinics at present than they were ten years ago, and there can be no question that this desirable state of affairs has been in part due to the strict enforcement by the immigration authorities of regulations prohibiting the entrance of trachoma into this country, and in part due to the stricter carrying out of rules of personal hygiene in schools and homes, and the greater care of eyes in the clinics. The terminal stage of trachoma is, however, still seen in adults who have lived in the city all their lives and who, so far as can be judged from the history, have contracted the disease during adult life. We naturally ask ourselves what characteristics these cases presented in their incipiency and previous to the stage of cicatrization. The immigration authorities, judging from such of their cases examined by us regard thickening of the conjunctiva, especially that of the retrotarsal fold, as the most characteristic symptom of incipient trachoma. The detection of slight thickening in this location, however, is sometimes a difficult question and a matter of personal opinion, and for this reason doubtful cases are isolated and observed for a period of some weeks before the final diagnosis is made.

In our series of cases a number that have showed clinically deep-seated follicles and a thickened retrotarsal fold have apparently recovered without a trace of cicatricial tissue in evidence.

Summary

1. Several processes in the conjunctiva of children, evidenced clinically by a more or less chronic thickening or follicle formation or both and formerly considered as a part of a chronic process called trachoma, may run a comparatively quick course to cure without the formation of cicatricial tissue.

2. Trachoma as it is described in its entirety is very infrequent at present in the public school children of New York City.

2. TREATMENT OF TRACHOMA AND ALLIED CONDITIONS AMONG THE SCHOOL CHILDREN OF NEW YORK CITY

a. PREVENTIVE TREATMENT

(1) *SPECIAL OPHTHALMIA CLASSES. SCHOOL CLINICS. SUMMER CAMPS*

By Anna I. von Sholly, Assisted by Caroline R. Gurley, Alice Kurtz, Ella Lipsky, Mary Schmidling, Theodora Herzig

SPECIAL OPHTHALMIA CLASSES

Practical measures on a large scale for the prevention of trachoma and other infectious eye diseases date from the beginning of the nineteenth century. They were first promulgated by the military authorities as rules

of hygiene for the army in order to keep in control the more or less severe epidemics of ophthalmias that played havoc with the troops. Since soldiers are recruited from and discharged back into the civil population, the next logical step was to extend the supervision of the state over the latter.

With the increased knowledge of etiology and prophylaxis of infectious diseases, measures more or less efficient are being taken in all advanced countries to ward off epidemics not only of "trachoma" but of all infections in public and in charitable institutions where numbers of people are collected together. Thus, in the twentieth century all nations that stand at the front have certain regulations for the inspection and care of their public school children. It is obvious that through the army and the public schools the state and municipality have the easiest and most direct control over a large proportion of the population. Each unit is in more or less close relationship with several units of the rest of the population through the family and neighbors.

The details of the method of control for the suppression and extermination of eye infections in the various countries, as well as the immigration regulations, are fairly well known and can be obtained from the attached bibliography. It is enough to say that as the result of the measures used "trachoma" is seen only sporadically in urban centers and not frequently seen in the country districts except in certain foci mentioned by Dr. Williams where the economic and hygienic conditions are of a very low grade. Large epidemics such as were known before the early nineteenth century do not occur. Small epidemics occur in an occasional institution or town but are quickly curbed. However, so long as these foci exit and migration continues to grow easier we cannot afford to relax our guard.

Dr. Williams has given the various theories of the etiology of "trachoma." Whichever be correct, it is agreed that contagion is carried through the medium of the conjunctival secretion. Moreover the logical deduction from our theory as formulated by Dr. Williams makes it incumbent on us to give much more consideration than hitherto to our methods of handling the acute and subacute conjunctival inflammations.

The general rules governing the prophylaxis of infectious eye diseases are those of segregation of the affected during the secreting stage and the enforcement of personal and domestic hygiene. The recommendations for body cleanliness, especially of hands and fingers, clean personal habits, individual bathing utensils (sponges, wash cloths, towels), the use of individual bed clothes, pillow slips, sheets, etc., are found in every book and article written on the prevention of these diseases and some of our methods of enforcing these recommendations are given in the succeeding paper.

The particular contribution which we feel we have made in the prophylaxis of "trachoma" is the establishing of special day classes in the public schools of New York City for children suffering from any form of conjunctival infection. Thus these children may be kept under control, segregated, treated and taught prophylactic hygiene.

The method of handling infectious eye diseases in all the cities of this country and Europe where attention is paid to the matter at all, is to exclude all children showing conjunctival secretion and simply advise treatment. A certain number of cities have an inspecting nurse or doctor to teach the child and its family the methods of transmitting infection. When the active secreting stages pass, the child is readmitted to school, and if not completely cured is told to continue under treatment. Of course this means that a number of subacute and chronic cases with secretion too small in amount to be easily detected may continue to be a menace to others.

In 1807 in England Mr. George Critchett, finding a large amount of eye infections in one of the schools, advised the establishment of "A ward or separate school," where these cases might be isolated, treated and still receive their education. In 1873 such a school was tried for a year with most satisfactory results. Not much was done after this on a large and more formal scale until 1888, when an isolation school for 400 children was opened at Hanwell. The surgeon in the school reported a decrease in ophthalmia from 33 per cent. in 1889 to 7 per cent. in 1893 and 5 per cent. in 1895. Later, as the result of the success of the school, provision was made to take care of the children of the poor law schools who were suffering from ophthalmia and two isolation schools were erected in 1903 and 1904 at Swanley and Brentwood, respectively, where the affected children are still being isolated, treated and educated. These schools are, however, *resident* ones.

In New York City there are more than 700,000 public school children. The 1912 Health Department Report showed that 33,860 of these children had suffered from acute contagious conjunctivitis during that year, and 15,245 from so-called trachoma ("granular lids"), which included folliculosis and follicular conjunctivitis. The larger part of the sufferers live in the poorest and the most congested parts of the city. Their surroundings and habits are such that each one is a focus of contagion to his neighbor.

Our school children have been under periodic and careful examination for eye diseases by the Health Department since 1902 and all children considered a source of danger to others have been excluded from school until certified cured by a physician. As far as her authority has carried her, the school nurse has forced every child diagnosed as suffering from "trachoma," follicular conjunctivitis, blepharitis, acute conjunctivitis, keratitis, etc., under medical care either of the family doctor or a public dispensary. To obviate any financial hardship, the Health Department established free eye clinics in the congested districts. The children not excluded, but under treatment are obliged to show the nurse at intervals a signed note from a doctor or a stamped dispensary card stamped with date of treatment to prove that they are under medical care. The excluded children have been supposed to report daily in the school nurse's office to show the progress of their case. As far as possible home visits for the purpose of instruction have been made by the nurse.

The weakness of the above system of handling eye diseases among

the school children—the prevailing system of all the large cities in this country and Europe—is that neither the Health Department nor the Education Department has full control over the children, and the valuable opportunity to concentrate on education in hygiene is not fully utilized. The exclusion of the infected child from school does not cover the situation. The feeble quarantine which it entails, does little else but call attention in a mild way to the contagiousness of the disease, and the need for treatment. The necessity for rapid cure in order to limit the period that the child is a carrier of infection and the teaching and enforcement during this period of personal and domestic hygiene are neglected.

The problem in New York City is not an easy one. There is a large foreign population, poor, ignorant, unclean in their habits, many parents willing and glad to use the excuse of "sore eyes" to have the child remain at home to help in the family work. The children are self-willed and indulged and when they refuse treatment are not forced by their over-ridden or deceived parents to submit.

Children suffering from folliculosis (up to the past year called trachoma by the school doctors) have been permitted to attend school regularly, provided they showed proof of being under a doctor's care. Between these children and the school nurse there has been a constant conflict. The child and the child's parents cannot understand enforced treatment where there are no or so few subjective symptoms. The child evades the unpleasant and irksome treatment, deceives the nurse, some even going so far as to buy date stamps and stamp their own dispensary cards. Where a particularly rebellious child has refused treatment absolutely the nurse has had to choose between allowing the child to have his will and thus to undermine her authority and that of the school, or exerting her power to exclude the child from school until he submitted—the very object the child frequently wished.

In the case of the excluded children, with their exclusion they pass out of the jurisdiction of the school and the nurse too. Only the actively infectious cases are usually excluded. These children may roam the streets at will, carry infection to all who come in close contact with them (as they frequently do in their play), for the 14 to 16 waking hours instead of the 8 to 10 hours otherwise spent out of school. These children do not necessarily place themselves under treatment, many are glad not to go to school. Girls take advantage of the plea of "sore eyes" to remain at home to help in the housework and are even encouraged by their parents. Boys have been started by exclusion from school on the way to become incorrigible truants. They acquire the street habit.

On the other hand, there is the financial hardship to the family when a child approaching 14 years of age is excluded from school and kept from getting his working papers through lack of required school attendance.

After a year's study of the problem, the workers at the Research Laboratory, co-operating with the Division of Child Hygiene, agreed that the exclusion principle was inadequate. They suggested to the educational authorities that experimental classes for children suffering from infectious eye diseases which ordinarily exclude them from school, be

opened in one of the most infected parts of the city. In October, 1912, two classes in close connection with a Health Department eye clinic were started in the lower east side at Hester and Allen streets as an annex to Public School 65. One wing of a vacated school building was fitted out by the Department of Education. On the ground floor is the playground, one flight up two clinic rooms and a demonstration room and above these two classrooms and a washroom. The classrooms each seat from 15 to 17 children. The washroom contains a porcelain wash basin with a goose-neck faucet worked by a foot lever so that the child touches no part with its hands. All washing is done with running water. There is also a bubbling drinking fountain of porcelain worked by a foot lever.

Three days a week a Health Department clinic is held in this building. All children with eye diseases from some sixty adjoining public and parochial schools are either sent or brought in squads by the schools nurse for treatment. The children who formerly would have been excluded are transferred without delay to the infectious eye disease classes. The principal of their respective schools is notified by printed post card.

The mild cases of folliculosis are instructed to use boric acid drops at home and are placed under the school nurse's observation. If these cases become marked or show secretion, they are referred back to the clinic. The marked cases of folliculosis are treated at the clinic one or more times a week, chiefly with bichloride of mercury rubbings. Other cases, such as blepharitis, keratitis, etc., needing treatment report to the clinic as heretofore. The children are given cards with the diagnosis stamped on it for the information of the school nurse who has typed instructions as to what course to pursue.

The children retained in the special classes are treated according to prescribed directions of the clinic doctor, twice a day by the special nurse assigned to duty at the Ophthalmia School. Medication formerly left to the child's discretion for use at home is thus given in the school. We now *know* that the child receives this treatment. If the child absents himself from school the truant officer is sent for him.

In addition to actually taking care of the eyes, the nurse in attendance teaches the children personal, family and social hygiene by talks, demonstrations and quizzes. In the demonstration room (furnished in simple fashion as a sanitary bedroom), demonstrations on domestic hygiene and the care of the eyes are given to the mothers. The children are taught the value of cleanliness. They are obliged to keep their hands and nails clean, and are taught not to rub their eyes with their hands or wipe them with their coat sleeves or cap. Their hands and persons are inspected daily, and as a reward for cleanliness an honor button is given them. At the end of the term the child who has the best marks for cleanliness receives a small manicure set. At the end of the school day each child washes his own desk and chair with soap and water. This is done chiefly for its educational value.

This school is unique among New York City schools in the matter of cleanliness. The clinic room, the corridors and stairs are mopped up daily. The waiting room and school rooms are wet-swept daily and

scrubbed three times a week. The toilets, banisters and side walls as high as the tallest child can reach are wiped down daily with a disinfectant. The chairs and desks in the classroom are wiped down with a disinfectant after the children have washed them with soap and water.

Needless to say that the clinic is run on aseptic and antiseptic principles. All possible precautions are taken to avoid carrying infection from patient to patient. A basin of antiseptic solution is on either side of the doctor so that the hands covered by rubber gloves can be easily disinfected between patients. This is done whether there is visible secretion or not. Medicine droppers are not allowed to touch patient's eyelids, and are always dipped in an antiseptic solution before being returned to the bottles. Bluestone pencils are not used from one patient directly to another, but are immersed in alcohol for a time before being used again. Pledgets of cotton wrung out of boric acid solution, one for each eye, are given each child to catch the excess of medication and secretion. These are thrown into an enamelled pail and burned later.

The nurse visits the home of the children in attendance and gives instruction to the mothers in their own homes. Printed leaflets in three languages, Italian, Yiddish and English, giving instructions on the care of the eyes are distributed to every new patient in the clinic (see p. 203).

Two teachers are in charge of the Ophthalmia School. The children range from 7 to 16 years of age and are divided into two classes of mixed grades, one of the older and one of the younger children. The children's eyes are protected from strain as far as possible. All work as far as it can be is oral and all reading and writing of very short duration *or omitted in severe cases.* Regular routine grade work is not made the object of the class. Since the majority of the children spend a comparatively short time in these classes, more stress is laid upon hygiene and the care of their health than on the "three R's." Such work as is given is rather for the purpose of keeping their minds and hands occupied. The school session is shortened, the morning session at 9.15 a. m. and the afternoon ending at 2.15 p. m. with one hour, from 12 to 1, for lunch. One-half hour in both morning and afternoon is devoted to recreation—play in the neghboring park in fair weather, story telling in bad weather. Last year's schedule is as follows:

9.15 to 9.30 assembly.
9.30 to 10 treatment and rest.
10 to 10.30 arithmetic.
10.30 to 10.45 spelling.
10.45 to 11.15 recreation.
11.15 to 11.45 English.
11.45 to 11.55 reading.
11.55 to 12 wash hands and dismiss.
12 to 1 lunch.
1 to 1.15 wash hands and rest.
1.15 to 1.45 recreation.
1.45 to 2.15 hygiene (Mondays), geography (Tuesdays, Wednesdays, Thursdays, Fridays).
2.15 dismissal to clinic for treatment.

Up to June 20, 1912 (nine school months) 312 pupils had passed through the Ophthalmia School. The various kinds of cases treated were as follows, namely, acute conjunctivitis (including acute contagion-conjunctivitis or "pink eye" and phlyctenular conjunctivitis), chronic conjunctivitis, "trachoma," follicular conjunctivitis, syphilitic, tubercular and phlyctenular keratitis and neglected protracted cases of blepharitis. When school closed June 28th there were about 25 children in the classes, with 17 ready for discharge. Only three during the year returned to the class after discharge with a recurrence of the disease. The shortest stay in the class was two days, the longest the entire nine months. Among the latter there were eight children with post-operative and cicatricial "trachoma" and pannus or ulcers.

The schools principals, school nurses and teachers are all very encouraging about the new classes. The school nurse is relieved of the unpleasant duty of hounding the children to doctors and dispensaries. She is relieved of the responsibility of the incorrigible who refuse treatment, the careless and neglected cases and the actively acute cases that carry infection—all of which are placed in the Ophthalmia School.

From the point of view of the educational authorities the child loses no school attendance, the authority of the principal is still in control since the child is not excluded, the arm of the truant officer is still powerful, school discipline is not relaxed and the child is not thrust out into the streets to become more familiar with bad company and bad habits.

From the health point of view the disease is attacked early and prevented from becoming chronic, kept under persistent treatment so that the cure is shortened and at the same time both child and parent taught how to avoid reinfection and spreading the disease to others.

We have made the recommendation that the Health Department request the Department of Education to establish one or two more of these special classes in cooperation with a Health Department clinic in the congested districts further uptown. We feel that with an intelligent nurse especially trained in eye work to oversee the school, there would be no danger of infection to other children if such a class or classes were held in a regular school building. The reason for this belief is based upon the following experience with the clinic at Public School 21.

SCHOOL CLINICS

While we were negotiating with the Department of Education for the formation of the special ophthalmia classes, an opportunity to open an infectious eye disease clinic in Public School 21, 222 Mott Street, was offered. This school is located in the congested Italian district somewhat remote from the Health Department infectious eye disease clinic then located at Gouverneur Slip. As an experiment, this clinic was opened in a room set aside on the second floor in the school building, January, 1912. We have no special infectious eye disease class in this building—there is no unoccupied room at present that can be used for this purpose. Clinics are held twice a week during the morning school hours, to which not only the children of Public School 21 come

but also children from the neighboring public and parochial schools. The children of Public School 21 come direct from their classrooms to the clinic room for treatment, losing only a few minutes from their school work. The children from the other schools come in groups with a "monitor," who is responsible for their arrival at the clinic and return to school. An exceedingly competent nurse was placed in charge to carry out the prescribed treatments twice a day as in the Hester and Allen Streets school. Children with acute conjunctivitis, phlyctenular conjunctivitis, follicular conjunctivitis, papillary conjunctivitis, in fact all children with conjunctival secretion who in Public School 65 clinic would be placed in the special class, either are excluded temporarily *to report to the school clinic twice a day for treatment* or, if secretion is slight, are placed in their regular classroom at a desk somewhat apart from the other children. Directions are given to the child and to the teacher how to avoid the conveyance of the contagion. The chair and the desk which the child occupies are washed with antiseptic solution after school. The banisters leading to the clinic room are washed daily with an antiseptic after each clinic is held. Through the exceptional personality and co-operation of the principal of the school, Mr. John Doty and the nurse in charge, Miss Schmidling, we have had absolutely no difficulty in keeping the children under control. The excluded cases, which are becoming fewer and fewer in number, report faithfully to the clinic twice a day. Miss Schmidling gives instructions to the children in personal and general hygiene, visits the homes and instructs the mothers. In spite of the fact that the school takes care of about 2,500 children and is not kept any cleaner than the average public school in New York City (with the exception of the clinic room, which is mopped up daily), we have had very few acute cases during the past two years. We have never traced an infection carried from one child to another in the classroom.

While we do not strongly recommend the clinic in the school alone without the classes for the excluded, it cannot be denied that it works very satisfactorily in this particular school. Without, however, the co-operation of the principal who has such a strong personal hold on his pupils as has Mr. Doty, and a thoroughly competent nurse with a personality that attracts children without loss of dignity or discipline, such a clinic might not be a success. To this clinic the mothers and fathers of the school children, the older and younger brothers and sisters, friends and neighbors are brought for treatment by the pupils. By treating them and allowing them to come twice a day if necessary we have gained a strong hold in the neighborhood.

SUMMER CAMPS

Since "trachoma" is one of the infections which bars children from admission to the Fresh Air Homes and Camps, and since no special provision is made for them, they have no opportunity for a change of environment and food except a rare trip to Coney Island with the family or a visit to a "country relative" in Hoboken or The Bronx. In ex-

cluding children from the Summer Vacation Homes, the broadest interpretation is given to the term "trachoma." No child with follicles, whether the eyes be in a secreting condition or not, is admitted. The justice of excluding these cases of folliculosis, now considered benign and non-communicable, is to be questioned. Of course, children with conjunctival secretion should be excluded. As I have said above, the children affected come from the poorest homes and the most congested parts of the city; most of them do not receive sufficient or proper food at home.

For a month in the summer of 1911 with the generosity of the A. I. C. P. we were able to send selected children from our clinic to a home set apart for them at Wilton, Conn. The result was most satisfactory. A nurse and a helper were with the children so that their treatment was carried out daily. Following this demonstration, it was hoped the Health Department would construct a permanent Summer Camp for "trachoma" and infectious eye diseases, but funds were not obtainable and the matter is in abeyance.

This past summer, 1913, the New York *Globe* made an appeal to its readers and through their generosity enough money was collected to send 29 children to a farm at Cudderbackville, Orange Co., for the whole ten weeks of vacation. The isolated location and the sanitary arrangement of the farm were well adapted to the demands of such a summer home. A Health Department Nurse and a volunteer helper were with the children all summer. Their routine treatment was carried on twice a day as in the special classes in New York. The doctor visited them at intervals. The children were out in the open air all day long, and bathed daily in the river at the foot of the hill. Each child had certain duties, such as making her or his own bed, helping in tidying up the room and washing his and her own clothes. The children were housed in three dormitories, each had an individual bed, with individual bags holding the clothes at the foot of the bed and, of course, individual soap and towels. They washed in running water of a small runlet in the open air. Cleanliness of the person and general behavior were marked daily on a bulletin posted in the hall and had a splendid effect in keeping up the eagerness to excel. All of these cases were chronic and had been under observation for a long time. Of the children who spent all ten weeks there, none gained less than five pounds in weight and a few gained as much as ten pounds. Those who were there a shorter time gained from 3 to 6 pounds each. As far as the eye conditions were concerned, while, in several cases the improvement was marked, in the majority we did not find any more noteworthy improvement while in the country than occurred in the city under *similar constant daily treatment*. We feel, however, that the summer work is important and worth while and ought to be made permanent for these reasons:

1. During the summer vacation, there is no one to urge the child to undergo treatment. He neglects himself and what has been gained from the winter's treatment is lost in the summer. Some of these

children who were in the country have been under our observation for more than three years, their condition before they came to us was chronic and has remained so. Each fall they return to school with a relapse.

2. The knowledge that faithful attendance at the clinic will mean a vacation in the country in the summer is a strong incentive to keeping under constant treatment. Since they are barred elsewhere, this is their only chance to get to the country.

3. While miraculous improvement does not accompany the sojourn in the country, the improvement of their general health, the better opportunity for the teaching of personal hygiene while in residence and the prevention of relapses play an important (though not brilliant) part in the program of prophylaxis.

4. Furthermore, neglected and running the streets of the city, these children are nuclei of infection.

Since the institution of the special ophthalmia classes, school clinic and summer camp, there has been without doubt a distinct improvement of the eye conditions in the lower east side of New York City. The large majority of patients now treated at the clinic since the opening of the second year of the special school (1913), are those of folliculosis, blepharitis (which is either on the increase or else more care in picking out the cases is taken by the school nurse), and pediculi of the eyelashes. This latter condition certainly seems to be on the increase, especially in one limited section of the lower east side. Only an occasional case of papillary conjunctivitis has been seen since the above measures have been instituted. Between 1910 and 1912 320 cases of papillary conjunctivitis were treated by us. The folliculosis cases seem milder, none has been sent to the hospital for infectious eye diseases at 118th Street for the operation of expression since October, 1912.

The fresh acute cases are seen early, are segregated and usually run a short course.

(2). PERSONAL HYGIENE AND FOLLOW-UP WORK

By Alice Kurtz, Assisted by Caroline R. Gurley, Ella Lipsky, Mary Schmidling and Theodora Herzig

Introduction

Early in the work of the Research Laboratory group on the preventive treatment of trachoma and allied conditions, home visits to patients attending our clinics were begun with several objects in view. These were mainly the examination and recording of the eye condition of other members of the family—both to determine the degree of home infectivity and to advise treatment, the taking of a more complete history than clinic conditions admitted of, the investigation of the environment in order to determine helping causes, the giving of instructions, both verbally and by demonstration, for the prevention of the spread of in-

fection, and *follow-up* work to be sure that the instructions were being carried out.

When the ophthalmia school was started (see page 182) practical teaching in hygiene, particularly the hygiene of the eyes, was a subject which received very special attention.

The work set forth in the following pages may be separated into two sections.

I. *Investigation*

The first question investigated was that of infectivity in the series of cases here presented. This is considered under two heads—Helping Causes and Home Infectivity.

1. *Helping Causes.*—At the outset inquiry was always made as to what was regarded by the family as probable source. Public baths; street playing, particularly ball playing; the schools, public and parochial; injuries; the visiting of relations in Europe having "trachoma;" and also the eye clinics themselves were frequently given as the source of sore eyes. Many children complained of never having had sore eyes until some time after being sent to the clinics by the school nurse. Other suggested sources were "catching cold"; a children's school in the country; libraries; school books and desks; and banisters in houses where there had been an epidemic.

Out of 117 families marked for general cleanliness but four are marked double plus, which shows that even minimum health standards in the home are not an easy matter. But 35 are marked plus, meaning cleanliness moderately good.

Of course the question arises, is it necessary to depend upon evolution here for results. We feel that this may be answered emphatically in the negative because of the hopeful results in habit-formation obtained by us in our ophthalmia school.

While in community or family work discouragement is nearly always present, in the work with the children in the special school there is nothing but encouragement for the friend with patience and perseverance who forgets discipline—so-called—to find the real discipline possible through confidence and friendship of the child. Teachers, nurses, visitors, all who come in contact with the child, must be imbued with the spirit of service and must recognize themselves as a part of this environment of the child.

The many problems that are literally thrown at the visitor in homes of sickness are at times staggering. One mother begged that her girl be cured of "bad blood" at 17 years of age, although she too had it when an infant. On examination this child was found to give a marked positive reaction for syphilis.

Improper Nourishment. Among the most serious hindrances to good physical condition in these children is unwholesome food. Almost every child in every family gets at least one penny a day because he cries or kicks for it. Many, of course, get more. One fascinating little fair-haired girl gets as many as 15 to 25 pennies a day because she knows

enough to ask her father for the money when he has customers around him, and the father seems proud of this fact. Many parents are literally afraid to refuse pennies and continue to dole them out even when the child has no appetite, rarely eats breakfast, and runs to the candy store as soon as he is out of bed in the morning.

One small boy of six was seen at the breakfast table one Saturday morning, still covered with day-before dirt, with sister and mother coaxing him to take an appetizer of whiskey to aid in the swallowing of a piece of fried fish, some coffee and bread. Nothing of this breakfast was eaten, but it was freely fingered. The visitor was told that he never had an appetite although he ate freely of candy.

Coffee and rolls for breakfast are almost universal from the baby of two to the grandmother who looks 80 but is nearer 50.

Lunch is frequently a "snatch" meal of a piece of dry bread or bread and butter or often something bought at a push cart. Some effort on the part of teachers and visitors is made to substitute the buying of fruit for sweet stuffs. There are, however, some mothers who prepare a lunch for the school children. The cleanest homes and the best foods are in orthodox families or the families with the highest ethical standards.

The evening meal is the one substantial meal of the day and is usually chicken or other meat, with vegetables boiled in the soup pot.

Lack of proper nourishment, abnormal amount of sweet stuff and a wicked waste of pennies, together with insufficient sleep, were found almost everywhere.

Overcrowding.—Of 116 families, on the basis of allowing eight persons to five rooms or five persons to three rooms, the number of families overcrowded was 67 and on the basis of allowing 1 person to 1 room (including kitchen) the number overcrowded was 111. Of the five families not overcrowded, one family only had more rooms than individuals, three persons to five rooms. Only two families out of 116 enjoyed the same number of rooms as there were individuals in the family. The other two least crowded households had, respectively, eight persons to six rooms and eight to seven rooms.

Sometimes it takes a very long time to get many of the details of the lives of the children. We had known one boy and his family nearly a year and had been called in by the mother to discuss a family controversy and the tendency on the part of her son by a former marriage to remain out all night, before we were casually informed by the small brother that, owing to the noise and general disturbance in this family of eleven living in four rooms, this older son had to put off his home study until after 11 o'clock at night.

Upon recommending a separate bed for one small boy, the visitor was informed that it could be managed. Some few days later the boy came to the visitor and said he wasn't going to sleep that way any more, he didn't like it. On asking why, the child said he was afraid of rolling off. It seems the separate bed had been made on the top of an upright piano.

Owing to the overcrowding of the rooms, to the noise, light, and disturbance on the part of their elders, the children do not manage to get to sleep much before ten and frequently are kept up later. One boy said he was awakened every night at 1 o'clock or between 12 and 1 by the boarder. He magnanimously explained that the boarder had to go and see his girl every night, as he was engaged to be married, and so the boy didn't mind.

Light.—The measurements of light, temperature and humidity were undertaken under instructions from Professor Winslow, of Columbia University, who has decided that the results shown in the light experiments are suggestive of wrong method and so they are left out of his report. The method used, however, showed at least the wide variations as, for example, the difference between 46½ in a dark kitchen and 81 at the front window on the first floor of the same apartment. The attempt was made to measure the light in the most used room of each of the 50 families visited. However, in this study no definite connection between dark rooms and "trachoma" has been shown.

Temperature.—The temperatures shown on the chart were taken in December and January. They imply at least that there is not much overheating. Of 41 families, no temperature was lower than 53 degrees. Between 53 and 55 there were three families; between 56 and 60, 12; between 61 and 65, 12; and between 65 and 70, 14. There was no case over 70. Thus, out of 41 families, 33 had 66° or under, 15 had 60° or under, and three had 55° or under. However, may not the desirability of some of these low temperatures be questioned, especially when noted in connection with poor food, or little of it, ill health, and insufficient clothing. Of course, in respect of both temperature and light it is largely only the mother and babies who really would suffer, as most of the others in the family are at home a comparatively short time, unless they are in bed. (For comparison of temperature and humidity measurements in 29 homes, see chart.)

Humidity.—The range of temperature and humidity may readily be seen on the temperature and humidity chart. The drop from air temperature, or difference between wet and dry thermometers, was measured by means of the Sling psychrometer and the actual humidity was obtained from the relative humidity table of Queen & Co., Philadelphia.

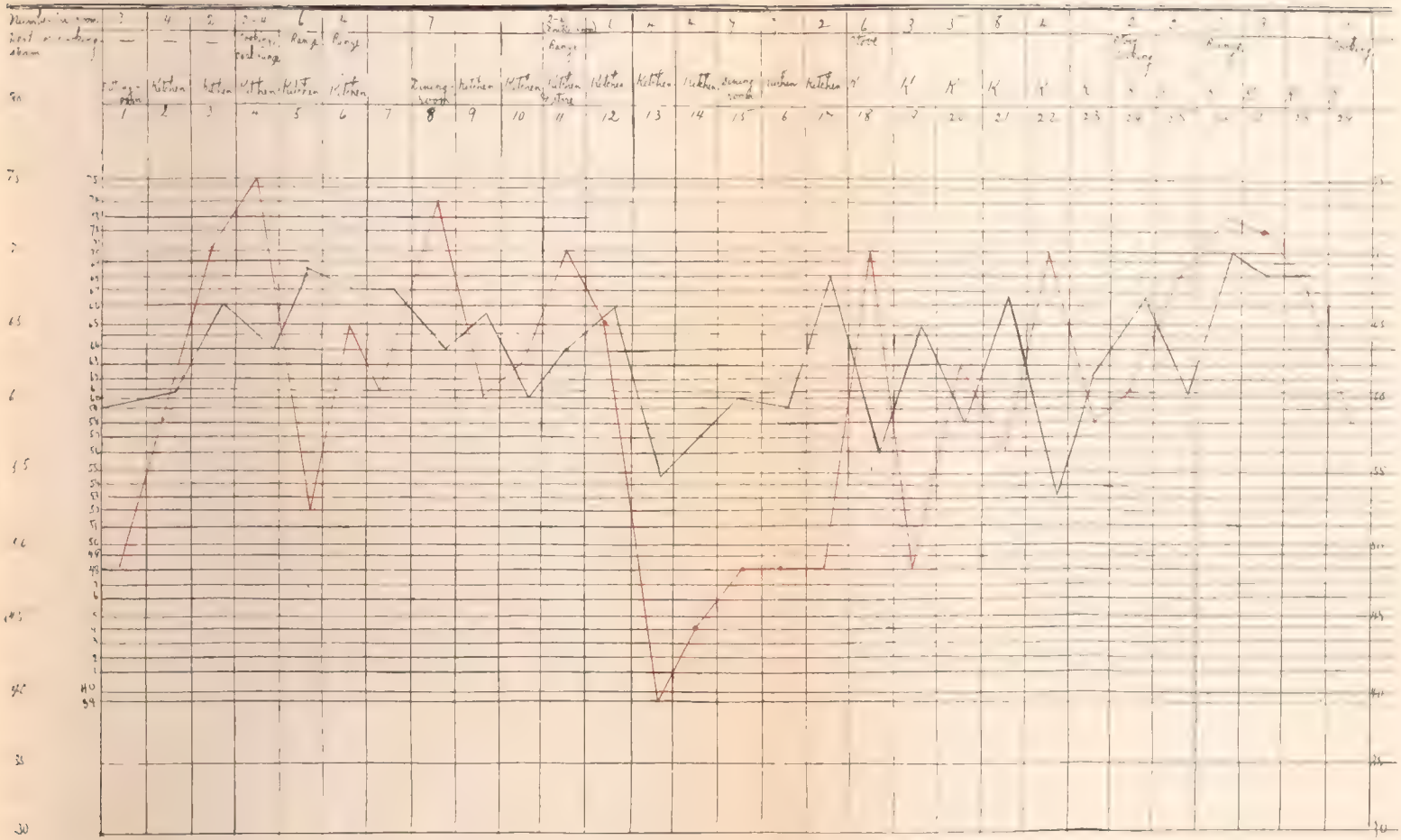


Chart showing range of temperature and humidity in [illegible] families

Toilets—Out of 158 families 13 only had toilets in their own apartment. According to law there must be one toilet to every two families.

On nearly all floors there are four families and two toilets. Many of our patients have 8 to 13 in one family. This number when multiplied by four may be imagined in connection with the use of two water closets. But taking the average number in the families as seven, it means that in one house, on one floor of four families and two toilets, there are 14 men, women and children to one toilet; that is, in the whole house of six floors there are 170 persons using the 12 water closets in this *one* house 365 days of the year, going through narrow, dirty and dark halls, stumbling over stairs, filthy and dark, and over stray cats and children. This is only in *one* house. This one house is massed up against another so much like it that the laws in regard to light and air are followed barely in the letter and not at all in the modern human spirit. One of the consequences of this overcrowding is that the children run frequently to the street for urination. Naturally the necessary washing after the use of the toilet is seldom if ever carried out.

In the home apartment there is water—frequently only cold—in the kitchen alone, *and beside the kitchen sink hangs the family towel.* The use of individual towels and wash-cloths and much soap and water is almost nil. Most of these people have not been taught the importance of personal hygiene.

The sanitary requirements of the city are for the most part carried out except perhaps in regard to care in avoiding odors. Stale odors and others were marked in many cases in the halls and toilets.

With the exception of a few houses—one in markedly bad condition —the toilets were fairly clean, at least superficially. The space occupied is very limited.

Employment—The largest number of fathers seem to be employed in sewing or pressing clothing or in selling. Of 60 families, 21 of the mothers act either as janitress or help at selling in store or at stand, while but 2 do home work—aside from housewife's work.

When sometimes the workers of the family, including mother and father, leave as early as 5 o'clock in the morning and the care of the children is left to the oldest child, it may be considered as little short of marvellous that the mother, or one of the older daughters, creates a semblance of order and cleanliness once a week, out of most chaotic and filthy conditions. Everywhere an effort is made to have a clean house on the *Sabbath.*

Income.—Although somewhat eager to note the income of families with "trachoma" it was found to be impossible in the time at our disposal or without special investigations looking to that end. While the questions of income and rent were put with as much tact as possible, much keener ingenuity was displayed in avoiding the answer. Such answers as "Wir machen *a* Leben," or "We have a couple of dollars," or "Well, we don't ask for no charity," or even a mere shrug of the shoulders were properly satisfying to the family but confusing to the visitor.

The means of existence ranged from none at all—where the workers were on strike, or where the principal occupation was "looking for a job," or because of sickness or seasonal occupation—to bank accounts of fair size. These latter were not acknowledged but inferred, and in one case proved, where the mother, who needed expert care quickly and had been sent to a hospital by the visitor, was refused as a suitable charity case by the City Department of Charities. It was found through letters from a bank that this family had an account of at least $2,000, while anonymous warning placed the savings at from $8,000 to $10,000.

Summary.—From the foregoing it will be seen that the chief causes regarded as contributory to the spread of "trachoma" and allied conditions are the crowded and unwholesome conditions of tenement-house life, which involve contact of normal children with those infected, and which lower the general physical condition and resistance of the child. These conditions are based upon insufficient income, inertia and ignorance. Parents frequently bear too heavy burdens in the care of a large family of young children during strikes, dull times, and "off" seasons in seasonal occupations. The cases of deliberate indifference and neglect on the part of the parents are few.

2. *Home Infectivity.*—The question of infectivity is such an important one and is in so confused a state because of the lack of a definite clinical entity, that two series of studies with regard to it were undertaken one by C. R. Gurley, which is considered on page 244, and the present one.

As our school clinics were in but two districts the children belonged with comparatively few exceptions to two races, the large majority being Jews and most of the others Italians. Out of 174 cases the number of children known to have been born abroad was about forty.

The two series contained a majority of families common to both, but a small number peculiar to each. The estimate of infectivity was, however, made upon a somewhat different basis in the two cases. For each series were selected families in which at least one member had "trachoma" and in which were at least several other individuals accessible to infection. In the first series only those members of the family were counted as infected from the trachoma individual whose cases were diagnosed originally as trachoma (see page 171). In the estimate which follows, however, cases diagnosed as doubtfully trachoma as well as those of "trachoma" (papillary conjunctivitis with follicles), were counted as originating from the individual having trachoma.

Tables were made including 64 families representing an aggregate of 577 persons. Of these families with an average of nine individuals each, 27 had home infection very probably and two had home infection probably. In the 27 households showing home infection, there were 211 individuals, of whom 68 were infected (see table). Calculating upon the basis of one member in each family having been infected from outside, we have 41 out of 184, as the number of home infections, or 22+ per cent.

TABLE SHOWING PROBABLE HOME INFECTION

No. of Family	Individuals in Family	Number Infected.		No. of Family	Individuals in Family	Number Infected.		No. of Family	Individuals in Family	Number Infected	
		Adults	Children			Adults	Children			Adults	Children
1	8	1	4	11	9	..	3	21	6	..	1 pos. 1 ?
2	8	..	2	12	7	1	1	22	5	..	3
3	9	..	1 pos. 1 ?	13	7	..	3	23	8	..	1 pos. 1 ?
				14	7	1	2				
4	11	..	4	15	4	..	2	24	9	..	2
5	8	..	2	16	8	..	1 pos. 1 ?	25	8	..	1 Tr. 1 Conj.
6	5	..	2	17	9	..	1 pos. 1 ?	26	10	..	2
7	11	..	1 pos. 1 ?	18	6	..	3	27	9	..	1 pos. 1 ?
8	7	..	2	19	7	..	3	..	..	..	..
9	6	..	3	..	..	..	..	..	..	..	..
10	8	..	4	20	11	..	2	..	..	..	..
	81	1	27		75	2	23		55	0	15

Total number individuals exposed to infection 184 (211 minus 27, i.e., minus 1 in each family regarded as source).
Total number of individuals infected 41 (68 minus 27), or 22+%.

The 27 families in which home infection occurred had an average population of nearly eight, yet the average space to a family was 3⅓ rooms, or about 2⅓ persons per room.

While the percentage of home infectivity resulting in cases diagnosed as trachoma seems to be low, the infectivity of acute conjunctivitis in the home and amongst playmates has probably a very high percentage. Thus in the home of H. H., all in the family numbering eight had "pink eye," with the exception of the two older sisters who slept together. No one knew how the infection was caught, but on questioning in regard to the first sore eyes it was found the baby of two years, was the first one. This small but active child had been playing with another child in the house who had "sore eyes," but who was now well. Further questions brought forth the fact that the boarder and all except the two elder daughters of the family used the towel then in evidence, hanging in the doorway between sitting-room and kitchen. These two daughters slept together and used one towel in common but kept it in their bedroom.

Teaching

In teaching, emphasis has been placed first upon immediate and follow-up instruction of the individual child and members of the family in all cases of secretion, and second upon preventive work with other members of family.

In the early days (winter of 1910-1911) of the clinic at Sixteenth street, Research Laboratory, each child on entering the clinic was observed by one of Dr. Williams' assistants and careful instruction given. Owing however, to the large number that soon came to the clinic, it was found impossible to give individual instruction and group instruction was supplied to those who came one-half hour to one hour before clinic. A Hygiene Club was formed and minute instructions given; the members promised to come to clinic with, and to keep, clean hands, neck, ears and noses, and to ask for and use at home, separate pillow, separate towel, separate soap, to wash under running water if possible, and in general to carry out the details of the Home Instructions given them.

Home Visiting.—In home visiting, in order to avoid implications of carrying infection from family to family, it was important that the nurse-visitor should not touch with unprotected hands the sore eyes of the child. Rubber gloves were worn and the hands were dipped in an antiseptic solution immediately before touching the lids. Thus sterile gloves or cotton wet in boric acid solution, alone touch the lids.

To get results, unless much more time could be given in the individual home, the demonstrations in cleansing the eyes in the home, were discontinued except in cases demanding immediate attention and in which the mother could not come to clinic or demonstration rooms, where the necessary teaching was provided.

Home visiting, as a consequence, *excluded* demonstration of cleansing of eyes in the home, except in the rare cases, and *included*, gaining friendly relations with mother or both parents; getting history of "sore eyes" in family and neighborhood; getting special information desired for special

study; demonstrating methods of utilizing home material and space for carrying out of suggestions of demonstration room; inviting mother to special demonstration room; following up of demonstration teaching; verbal teaching of prevention and control of infection in the home; never failing reminders of the infectivity of secreting eyes and constant emphasis upon cleanliness; following up members of family who did not come to clinic; and keeping all members of infected family under observation.

The fact that this work of preventive teaching may accomplish wonders may be illustrated by the case of a centre of infection in Broome street. One child was sent to the clinic by the school teacher and had not appeared at the clinic. On notification, the "trachoma" nurse went to the house and interested the mother in bringing her child to the eye clinic. No other children in the family had an eye infection and inquiry brought forth the fact of several sore eyes in the neighborhood.

Six children were then called upon, all of whom named one child as having the first sore eyes in the neighborhood. This latter boy had been previously a case at our own clinic, but was now well. The mothers of these infected children kept them home from school and they played freely with many others in the streets. Altogether, those known to have come from this particular *playmate* source numbered 7.

The playmate source of infection, whether at home, at school or on the street, seems to be one that needs special attention. All of the blame of infection cannot be laid on the family towel as long as children love to take care of the tinier ones, or love to show their affection for their playmates.

A great deal of effort has been expended in striving for some control over family habits, the majority of homes visited possessing a family towel, dank and mysterious, but wholly precious. Suggestions as to the necessity of separate towels, face cloths, soap, burning of eye cotton, faithful cleansing of hands and eyes, were met with a ready acquiescence—seldom fulfilled—or with a hopeless shrug of tired shoulders.

One or two of the families, however—after the whole family had become infected—had learned certain details of care by bitter experience, and so realized the importance of our efforts to instruct them. Thus Mrs. —— asked why all this effort and teaching of mothers and children had come so late. "Why," said she, "did you not try to do this seven years ago, when we first had sore eyes? I myself, many a day, have sat on one of these benches and told mothers to take care of their children's eyes. I am no bacteriologist, I cannot even write my own name, but seven years ago, I thought the mothers ought to have been taught and ought to have had the leaflets you are giving to-day."

In spite of the usual inertia, the word "catching" was in nearly all cases an open sesame to interest and friendliness. The difficulty of changing the attitude or actions of some of the families may, however, be observed in the case of Harry K. For two years this family was visited frequently, the mother attended demonstration classes, and Harry got worse. Even coming to the clinic several times a week did him no permanent good. He was sent to the hospital until the secretion, which

constantly poured from his eyes, was taken care of. He was returned home improved, but was soon again in the same old state.

It was a simple case of home neglect and mental or perhaps physical inertia. However, it may be mentioned that his mother was almost constantly ill. She was sent to a hospital and after a sojourn of several weeks, she came home, moved into lighter rooms, and in general took more interest in the life of her family.

But the real change became apparent when this child was made to come to the Special School regularly. He was treated two or three times a day or as often as the doctor thought necessary. He was made to become as clean as was possible at the school. His *nose* and *eyes* and *fingers* were under constant supervision. His mother actually cooperated, when she became stronger, by insisting upon his attendance at school without the help of the Truant Officer. She also kept him in fairly clean blouses, something that had been unknown before. It is believed that she even made him take his medicine at home, besides probably giving the "tonic" to all of the children in the family—as they were constantly asking for more.

Harry was always dull, stupid and obstinate; his physical handicaps making him more or less lifeless. He had been out of school for months and as he could scarcely see, he sat in the back yard most of the time playing with the baby. The life in the school, however, and the daily walks to the park with other children soon changed him into a much livelier boy. He became so bright that he is now in the care of the Gerry Society and has made a fine start toward prison, unless taken in hand by a modern psychologist.

In some few families, some special effort was made to help the families themselves in finding separate towels. Fairly good towels would be pulled forth from a weird collection of rags; a nail, or nails, was found somewhere and hammered into the desired spot.

Clinic Instructions in Prevention of Infection—Such a simple operation as washing the nails and arms, was found to be fraught with many difficulties. Time after time it had to be taught in minutest detail and with never-ending patience, and frequent comparisons made with instructor's hands and nails—particularly as to color.

Much attention was paid to cleanliness of nails, without aid of brush or other nail apparatus, getting the soap—preferably "green" soap—well into nails and using the finger nail of the other hand as a cleaner (see photograph). Talks at the same time were given on the purpose of the nails, and on drain pipes and the special need of, and particular pleasure in, having the individual soap and towel, and hands that showed care.

Although washing the hands before meals is a religious and health law with the majority of our patients, the methods in use leave much to be desired, as the family towel mutely but eloquently shows.

As the result of this one simple operation is sometimes momentous in the prevention of disease in the home and in many occupational diseases and as it is extremely difficult to teach the adults its significance.

and still more difficult for them to acquire strange habits, it would seem important for the state to recognize the desirability of forming habits of clean-ness in the young. This will be done, perhaps, in the day the school can supply what the state should provide—home training and environment for the children of the untaught and the indifferent. Habits may take long in the forming, but the results are assured.

The printed "Home Instructions" given below were found lacking in some details which were filled in by the instructor.

DEPARTMENT OF HEALTH

The City of New York

HOME INSTRUCTIONS REGARDING CARE OF "SORE EYES"

GENERAL

Before attempting to cleanse the eyes, wash hands and finger-nails, using warm water, and plenty of soap, particularly in nails.

Wash hands as thoroughly *after* touching the eyes.

Wash under running water if possible, but if basin is used, scrub basin with soap (or soap powder), hot water, and brush before and after using.

Have separate towels for each person in family.

Have separate wash-cloths for each person in family

Have separate pillow-cases for each person in family.

Have separate handkerchiefs for each person in family.

Those with sore eyes should not sleep in same bed with others in family.

After using, pillow-cases should be aired, in sun if possible, and then covered or kept from contact with other pillow-cases.

After using, towels should be aired, in sun if possible, and so hung that they do not touch one another.

All towels, handkerchiefs, pillow-cases, wash-cloths, used by person with sore eyes should be changed, washed, and boiled as often as possible, but at least twice each week.

It is important to keep child's nose clean by blowing nose at least twice a day regularly, morning and night.

To clean nose, apply some vaseline in nostrils, on cotton wadding wound about a tooth pick or burned match.

Have windows opened at night. Give simple food to eat. See that bowels move daily.

Keep child's fingers and finger-nails clean.

TO WASH EYES

First wash hands as directed above.

Have boric acid water and clean absorbent cotton (from drug store) or clean (that is, boiled) soft, old white rags.

Use one piece of cotton at a time, and on one eye only; wipe away secretion (matter) carefully from eye, and eye-lashes; burn cotton at once.

Wash eyes every hour, or oftener if necessary, while child is excluded from school.

As long as child is under treatment at dispensary, eyes should be washed at least five (5) times each day.

TO PREPARE BORIC ACID WATER

Buy boric acid powder. Add one (1) heaping teaspoonful of boric acid powder to one (1) cupful of hot water. Boil in covered pan. Put in clean bottle when cool. Shake thoroughly. When cool, sediment, if any, will sink to bottom of bottle. Do not shake again. USE WHEN COLD.

TO USE MEDICINE

First wash hands as directed above.

Then wash eyes as directed above.

Have clean medicine dropper.

Have piece of moist cotton to catch overflow, do not use handkerchief.

Have child's face upturned and eyes opened.

Drop medicine in corner of eye, nearest nose.

Have child close lids and roll eye-ball.

Wash away overflow with moist cotton, do not use handkerchief.

Burn cotton at once.

Wash hands and finger-nails again.

Clean medicine dropper.

TO CLEAN MEDICINE DROPPERS

Remove rubber top. Boil for about one (1) minute, both glass and rubber parts in water containing washing soda. Rinse in clear water when cool.

Keep all eye utensils clean and dry, and free from dust.

In some contradistinction to the difficulties found in some families the real helpfulness and interest shown in many families may be mentioned. Some of the difficulties in changing conditions and habits may be understood when it is pointed out that only 3 out of 117 families were marked double plus for cleanliness and order and only 25 single plus, i. e., as having the ordinarily desired cleanliness and order without a question-mark.

In each of the three families showing unusual cleanliness at each visit, separate towels were already in use and separate beds in two. A high degree of intelligence also existed here, with bodily health.

Mental Attitude.—A rating on the Mental Attitude exhibited by parents is rather a difficult performance. But an attempt was made, as elsewhere, to show with plus and minus marks, the attitude and personal responsibility of the parents.



Separate beds and towels in corner of model flat.

In the chart kept of these points the percentage of indifference is remarkably low, only four cases on the part of the mothers, showing inertia or indifference. Considering the extraordinary difficulties under which the majority of these mothers labor, together with their physical ailments, the personal responsibility and interest shown is very high. Of these 51, thirteen, or about 25½%, showed marked initiative. This is very encouraging for future home work. Under receptivity, the majority are marked fair.

Of the three difficulties, overcrowding, too much work (including illness) and poverty, overcrowding stands out as the most positive, aided largely by a general lack of order. (Note "Helping Causes.")

Demonstrations and Mothers' Classes.—Owing to the difficulties of teaching asepsis in the home and the ineffectiveness of attempts by the mother to follow verbal instruction, it seemed important to establish a demonstration or teaching centre for the mothers and children.

With the most active and cordial co-operation of Miss Kittredge, founder of the Association of Practical House-Keeping Centres, more thorough teaching work was started at two of the Model Flats of the Association, one (see photograph) on Henry street in the Jewish district, and the other in the Italian quarter. Because of lack of time, the work in the Italian quarter was later discontinued, but the model flat on Henry street (photograph) was in use until the old school at Hester and Allen streets was fitted up for Special Class and Clinic work. In this latter building a Demonstration Room is now provided. The Demonstration Room contains 2 beds, spread as if ready for use, the smaller a trundle-bed, which, for economy of space, fits under the larger one. In addition there are a wash-stand with basin, pitcher and soap-dish, surrounded by a folding screen, a table and several chairs. All the furniture is white-enameled and the basin and pitcher are of white enamelware (unbreakable). Into the frame-work of the screen, screw-hooks are inserted and on these hang the separate wash-cloth and towel for each member of the supposed family. By means of a long, horizontal strip of tape attached to the washable material with which the screen is covered, the comb and tooth-brush of every individual are provided with a place, as in a rack, near the wash-stand.

During the winter of 1911-12 the mothers and their infected children were invited to the Model Flat where the kitchen and sitting room were used for the purpose of demonstration. Incidentally, the demonstrations were not limited to strictly technical lines. In the cold weather Miss Kittredge provided hot cocoa and biscuits to be served in the Model Dining Room to our mothers and children. This latter pleasant arrangement had later to be discontinued, because of the numbers it was found necessary to teach at one time. It has, however, been recently renewed in connection with the Ophthalmia School, where demonstrations are enthusiastically attended. At these the serving of tea is a feature.

The advantages of having a kitchen much the same as the kitchens in the district, were evident when it came to disposing of cotton that had touched the eye and when the arrangement and care of basins (and

other utensils), soap, and individual towels were considered, especially as the mothers were present at the classes.

In the Demonstration Room—or Model Flat—the actual "Home Instructions"—p. 203—were carried out. Demonstrations were given in the care of hands and nails, washing of eyes, method of dropping in medicine, care of utensils, handkerchiefs, towels and pillow cases, burning of eye-cotton, making of boric acid solution, etc. The most compact arrangement for having separate beds and the best method of caring for a number of separate towels in a small space were shown in the sitting room (see photograph). To these were added, at the Special Class, simple experiments, and talks and quizzes in preventive hygiene, with practical work by the young members of the classes, criticized by their confrères. The demonstrations showed just what is possible in the home. The "trachoma nurse" then visited the family, after detailed instructions had been given to the children and mothers, to see that separate towels were used or to have the mother give an unexpected demonstration.

Special Class Instructions.—Upon the establishment, through the co-operation of the Board of Education and the Health Department, of a Special School of two classes and an affiliated clinic for children, excluded from the regular school on account of contagious eye diseases (see p. 185), the work of instruction in hygiene was transferred to it. The great difficulties were largely overcome by the faithful watchfulness on the part of the teachers, Miss Mansion and Mrs. Smith, under the direction of Mr. Bulkley, principal of P. S. 65. Without their interested and sympathetic co-operation, the efforts at habit formation would have been largely unavailing. It was necessary to counteract many lifelong tendencies by patient reiteration of the desired substitutes.

The results of the combined efforts of those in charge were a constant source of pleasure to them. The children themselves soon detected carelessness on the part of their confrères and even howled for the opportunity of washing their hands and nails, for the use of their separate paper towels, and for the opportunities of "helping" in the clinic. One physician satirically suggested that they "cried for it." This was almost literally true. Examples of this were frequently seen and heard, but especially in the "Surgical Cleanliness" preparation necessary before applying their hot application to their own eyes. Any little slip in "technique" here brought forth prompt shouts of objections from the other small patients.

In connection with the "Special Class" or Ophthalmia School are given demonstrations and talks on prevention of contagion and on personal hygiene. Every child who enters the clinic except in "rush hours" is ensared into taking off coat, rolling up sleeves and attempting to change the color of hands and nails to more nearly the color of his instructor's hands and nails, while a little lecture is given on the connection between diseases, especially his eye trouble, and dirty hands and towels. Occasionally one or two have an "afraid from it," but the combination of a little tale about microbes, a little ball of green soap just for



Washing of hands.

himself, with a separate paper towel, and comparison between his own black nails and the white ones shown him, usually wins over even the most obstinate. In the clinics, also, is shown to child and parent or visiting relative, the proper methods of using medication and how to avoid touching the eyes with the fingers.

As a single example of what may be accomplished through the special class may be mentioned the case of a boy who showed little or no result after a year and a half of effort on the part of the visitor to the home, but quite marvelous improvement after a few months of the constant supervision afforded by the school.

In connection with the class rooms of the school the wash room is the centre for individual instruction in reasons for coming to the class, how to take care of the apparatus shown—the wash basin and drinking fountain have *pedal* attachments—how to wash hands and nails, and what to do at home in order to help himself and to prevent others getting the disease. The necessity of appearing at school every morning with clean hands, neck and ears, and just how and when to wash eyes and face, are emphasized.

With certain cases attending the Special Class special intensive work was done. It is interesting to note that of 51 children who received individual instruction, more home visiting than could be done upon patients as a whole, and clinic demonstrations, all but 6, or 88%, are marked on the Hygiene Chart as showing positive effort on the part of the children to put into daily practice the instruction received in class and home. The six are marked fair; no one is marked as failing.

The Committee on Prevention of Blindness of the Russell Sage Foundation have very kindly lent us large educational screens depicting the dire results of the family towel, and other causes of "Trachoma." The studying of these screens is part of the education of each little pupil-patient. One screen, an awful example of the roller towel, actually causes shivers of something to be manifested when the new patient is asked if he thinks that is the proper thing to use against his face. It seems so easy to agree that this towel is a carrier of disease—or rather that it might become so if in use—just as the screen says, but it is so hard to see the blackness of the home article.

The teaching of general cleanliness was also impressed upon the children through the care of their desks. Each was given a piece of cheese cloth and a basin. With these and copious water and a "ball" of green soap, every afternoon before leaving each desk was washed. (See photograph.) The cloth and the basin were kept clean by the child who was intrusted with them. This work did not affect the routine of school cleaning by the school helpers, however, which included the washing with carbolic solution at regular intervals, of the desks and all parts of the school touched by the children's fingers.

Senior Class Instruction.—Half hour talks on hygiene were given twice a week. These included some reference to the anatomy of the normal eye and very slight reference to the general anatomy. Drawings and prints only were shown. Practical talks were given and practical



Children washing desks.

results were expected along hygienic lines. Outlines for the daily care of the body were required to be written out, and quizzes, many and searching, were a real delight to the nurse-teacher as *mental* results seemed to come with much promptitude and ease, whereas physical results were at times discouraging.

Finger-print impressions were taken on agar-agar and showed many brilliant results in growths of vari-colored bacteria and molds.

The interest of the children in workable hygiene in this school seems to me to point the way to wonderful possibilities.

Junior Class Instruction.—The same amount of time was given to the little ones and they showed just as much interest and perhaps more serious attention. Here, of course, the talks were largely questions and answers on health habits. Mollie, aged six. frequently interrupted with. "I should say to my sister, 'Separate soap and separate towel.'" This class, however, took just as much interest as the older ones in the agar experiments, and were desperately serious in the tale of the stove that "went out" because it had too much food thrown in and could not get rid of the waste, and in the comparison with the stove that could do so much for people when it had a good fire burning in it. They knew a lot about stoves!

The effective screens of the Committee on the Prevention of Blindness were used here too.

A real effort was made to substitute new for old habits, and some actual results were obtained, although probably but temporary.

Honor-Roll.—To those who carried out the teachings in cleanliness for a month, keeping day by day up to a certain standard, was given, with due ceremony, an enameled button on which was printed "Clean Hands, Clean Habits, Clean Homes." Besides this, all children were daily marked for cleanliness.

Some Opportunities.—On passing in review the many hindrances to a healthful and improving life for out patients, several suggestions present themselves. Instead of shorter school hours why not longer school hours? Not longer hours at a desk, but longer hours under the control of the school, spent in *play* in the open air, in *rest,* in domestic science and manual training. In connection with their instruction on the prevention of infection, both boys and girls could be taught how to wash and boil clothes properly, and the necessity of so doing. The boys were always interested in the possibility of making a screen such as the one used in the "model" demonstration flat, and the arrangements necessary for keeping individual towels. Such interests might be utilized in their manual training work. Some of the children now come to school at any hour from 7.30 a. m. on, only too glad to get away from an unwholesome atmosphere of 9 persons in 3 rooms or 11 persons in 4 rooms. Perhaps with this lengthening of the hours spent under intelligent supervision will come legislation advancing the age limit at which a child may leave the only teaching he gets. He leaves school now at the most unstable period of life.

The opportunities of teaching clean habits and other health habits have been mentioned. In this respect a decided advantage might be gained could other visitors to the home, especially the ethical teacher and Hebrew reader, be enlisted to co-operate in giving the never-ending reminders.

The lunch hour could prove a splendid source for suggestive teaching. In most schools and homes it is now a time of trial for all concerned. What it is now and what it might be is a most important problem.

And is it necessary to get tuberculosis before the open air school comes into general use? Probably all of these children under observation in our clinics are anemic or at least sub-normal.

Conclusions

The burden of the large reduction in the number of bad contagious eye cases should be laid to several factors, the most prominent being probably the different methods followed in the eye clinics. The factor next in importance is regarded as being the marked development of "follow-up" work in connection with the clinics, including special attention to "first time" acute cases.

The follow-up work should include at least the following aspects: (a) the teaching, in the home of every infectious case, of the ways of avoiding the spread of infection, and of how to control the condition in the member affected, and (b), the securing, through home visits and through demonstrations and talks in connection with the clinics or the special class, of more hygienic habits of life.

Co-operation

Throughout the work we have received much cordial co-operation from many who have been interested in its progress.

Dr. R. T. Wilson, Superintendent of Health Department Hospitals, gave us valuable advice and material assistance in the arrangement and furnishings of our clinics and demonstration room.

Mr. Sparre, of the Brevoort Mission House, and Miss Kerr, of the Department of Health, have taken much pains in arranging for summer outings for our children; while Mrs. M. M. Marre and Mrs. Weil aided financially in sending some of the children for summer vacations.

A friend of the clinic arranged for a splendid Santa Claus celebration in our Christmas season.

Mr. John Doty, principal of P. S. 21, who suggested the value of the school clinics, helped in many other ways. We also owe a great deal to him and to Mr. Kidd and Mr. Bulkley of P. S. 65 for their active aid, as we do to Miss Mansion and Mrs. Smith, who have valiantly fought life-long habits while substituting new ones.

To Professor Agide Pirazzini, of the Bible Teachers Training School, we owe the translation of the Home Instructions into Italian, and correction of the proofs. Miss Mishulow, of the Laboratory, very kindly undertook the translation into Jewish, while, through the overtures of

Mr. Frank, Superintendent of Beth Israel Hospital, the Jewish translation was typewritten and printer's proofs corrected.

To Miss Kittredge our thanks are due for the use of unusually favorable demonstration rooms, while it is impossible to enumerate all that we owe to the Committee on Prevention of Blindness.

Mrs. Weil interested a group of young Jewish girls in supplying the "Honor Roll" Buttons, which were given to those who made an effort for "Clean Hands, Clean Homes, Clean Habits."

Miss Sharp, of the Beth Israel Hospital, took a personal interest in those members of our patients' families who were referred to the Hospital or to its Social Service Department. Mr. Frank, superintendent of the same hospital, aided in many ways.

Many philanthropic and social agencies cordially co-operated in the care of our families; for example, the New York Department of Charities and Corrections, the A. I. C. P., the United Hebrew Charities, and the Beth Israel Hospital.

The Educational Alliance, the College Settlement on Eldridge street, and the Clark Neighborhood House were interested in getting in touch with the boys and girls through classes and clubs and motion pictures.

Where destitution or some particular need, such as milk or eyeglasses, was found or suspected the United Hebrew Charities or other relief agency or interested Hospital was notified and cheerfully responded. It may be added here that where Big Brothers or Big Sisters were thought to be part of the treatment of any member of the family, the Educational Alliance, the University Settlement and other Neighborhood Houses promised their aid. The one important difficulty here was the over-demand for help along this line.

In one instance the Committee for Prevention of Cruelty to Children most kindly sent a visitor to give aid and counsel to a mother distracted by her oldest boy's midnight habits. The Educational Alliance sent tickets to their "moving pictures" in order to get some of our difficult boys interested in going to the Educational Center.

b. CURATIVE TREATMENT, INCLUDING VACCINE TREATMENT OF INFECTIOUS EYE DISEASES

By Herbert W. Wotton, and Anna I. Von Sholly, Assisted by Caroline R. Turley, Olga Powitzky and Percy Crane

The curative treatment of trachoma and its allied conditions or, if we please, of those affections of the conjunctiva which are characterized by the formation of follicles or by hyperplasia or by both, and which, by their continuance or exacerbations, may be considered as likely to eventuate in cicatrization and involvement of the cornea, falls conveniently under five headings: (1) hygiene, (2) medicinal treatment, (3) mechanical treatment, (4) surgical treatment, (5) vaccine treatment.

The hygienic and prophylactic measures employed by us are given by Dr. Von Sholly, Miss Kurtz and others (pp. 182-213).

In regard to our curative treatment we may state that, in general, the mechanical and surgical methods and the drugs employed have been those described in every text book. The only new treatment tried by us is that of vaccines in certain selected cases. This will be dealt with later.

We wish, however, to emphasize particularly the following points in our handling of the series of cases described in the previous paper (p. 171).

1. The large number of cases which were treated from or near the beginning of the disease.

2. The frequent change of treatment in the subacute and chronic cases.

3. The absence of drugs and surgical treatment in a large percentage of cases formerly supposed to need them.

The applicability of the various methods of treatment to the four groups of conjunctival affections already described, may be seen by the following brief statements of our handling of each group of the results obtained.

I.—*Folliculosis* (Follicles scattered over an otherwise apparently normal conjunctiva). In *mild* cases no treatment was used except boric acid solution (3 per cent.) at home and an insistance upon the rules of personal hygiene. These cases were sent to the clinic once a month for examination, or sent by the school nurse within that time if inflammatory symptoms supervened. The great majority of these cases, so far, have at present normal conjunctivas, and the others are practically normal (Table I). In more marked cases bichloride of mercury (1-500) rubbings (with cotton swab) have been employed once a week. In a few of the more chronic cases sulphate of copper stick has been *infrequently* used. We have not found it necessary yet in this series of some 2,000 cases of folliculosis observed for from 2 to 4 years to use expression. If the operation of expression is performed, it should be

done with a clear understanding that the disease attacked will not eventuate in deterioration of vision and that the operation itself may be followed by unpleasant consequences to both conjunctiva and cornea.

II.—*Follicular Conjunctivitis* (Follicles scattered over a non-thickened inflammatory conjunctiva). In these cases, if the superadded inflammation is acute and attended with secretion, argyrol, 20 per cent., or 1 per cent. silver nitrate solution, has been first used. Later, bichloride of mercury has been rubbed on the under surfaces of the lids. With Morax-Axenfeld diplococcus infection, sulphate of zinc (0.2 per cent.) has been used. In the few sluggish cases the above treatment has been alternated infrequently with the blue stone pencil. The great majority of these cases have recovered without the last named treatment.

III.—*Papillary Conjunctivitis* (formerly called acute trachoma). Argyrol, 20 per cent., or silver nitrate solution, 1 per cent., has been used in the beginning with these cases, when there is usually more or less marked secretion. Later the blue stone pencil, alternating with tannic acid (40 grains to an ounce of glycerine), has been employed. From time to time in the more chronic cases all medicinal treatment (except the home boric acid washings) has been suspended for a variable period. After this, a certain number of the cases seemed to respond more favorably to the renewed medicinal treatment. If we could have such cases as these under hospital care, as Cohen and Noguchi had their cases, which recovered after only boric acid and cold compress applications, simple hygienic proceedures would probably be sufficient to allow a great number of them to recover after several months.

None of the cases of papillary conjunctivitis over whom we have had control have been operated upon.

IV.—*Old Trachoma* (Cicatricial formation with or without pannus). In our series of cases among the school children we have as yet had no cases passing into the cicatricial stage. Among our pannus cases only eight have developed this condition after being seen by us. Two of these left us, and all of the other six, *none of whom have been operated on,* have now clear corneas, except one who has a narrow rim of inactive haze over the upper part of each cornea. These cases were treated medicinally in the usual way, and certain of them have been treated by vaccines. They are being carefully watched for further developments.

In the adult, of course, the condition of cicatrization with its danger of probable supervention of corneal involvement, is complicated by the fact that the patient is usually a wage-earner. As Boldt very truly states, our estimation of the value of a method of treatment must depend largely upon its applicability. These chronic cicatricial cases, as a rule, and in New York almost invariably, occur among the working classes, who have neither the means nor inclination to pursue any palliative or expectant plan of treatment, and for economic reasons it is in these cases, in the opinion of one of us (Wootton), that excision is preeminently indicated, and that in all cases in which the cornea has actually become involved, excision should be performed at once.

Excision, however, does not always mean cure, since a certain percentage of individuals in our old pannus case have shown a continuation of the pannus in aggravated form after the operation of excision.

Acute Catarrhal Conjunctivitis.—When our laboratory workers found that hemoglobinophilic bacilli of the same type were found in many of acute, subacute and chronic conjunctivitis, we made special efforts to follow up in our treatment all of the acute cases of conjunctivitis in the schools and in the homes of the children in order to help eliminate this form of infection from chronic cases. Now, whether largely as a result of this or not, we cannot yet tell, we have very few of the chronic papillary or follicular conjunctivitis cases.

Vaccine Treatment.—For the report given here of vaccine treatment in these cases Dr. Von Sholly is responsible.

The following is a tentative report on the results obtained from the use of subcutaneous doses of various vaccines in a few cases of eye infections at the Health Department Clinic for Infectious Eye Diseases at P. S. 21 and P. S. 65, Annex.

The favorable results obtained with tuberculin in phlyctenular and ulcerative keratitis confirm the reports of other investigators. The rapid cure of styes and infected meibomian cysts with staphylococcus vaccine is in accord with what one might expect. The use of streptococcus vaccine (suggested by Dr. Williams), however, for pannus and interstitial keratitis, is, as far as we know, new. Since it has been used less than three months, no positive statements can be made. The cases are reported for what they are worth. The marked improvement, especially in cases I and II, which had previously been very recalcitrant is most suggestive and full of hope.

Streptococcus Vaccine, Pannus.—Case I.—J. P., age 11 years. Duration of sore eyes not known, but under our observation almost two years. Her conjunctiva during this time presented deep seated follicles on both upper and lower lids, at times with and at times without inflammatory symptoms but no corneal involvement, until eight months after we first saw her, October 29, 1912, pannus developed in right eye. The Von Pirquet test made at this time was negative. Daily treatments of atropine, hot applications and ung. hydrargyri flav. ox., 2½ per cent., into conjunctivial sac, were given, with either bluestone or corrosive sublimate (1-500) rubbings twice a week. After a little less than three months' treatment, the right cornea became clear again. March 4, 1913, 1½ months after this, pannus developed in both eyes, but was more marked in right. A Wasserman test was negative (May 27, 1913). The same treatment as above was instituted, but very little improvement was noted. June 30, 1913, the child was sent to our country camp, but after 10 days returned on account of a death in the family. From then until school opened (September 16, 1913), she was seen only twice and had marked pannus in both eyes, covering half of cornea, and pronounced photophobia. There was also a scaly papular eruption all over her face which had been present all summer. On September 29, 1913, 25 millions streptococci (stock vaccine) were injected. October 3, 1913, 50 millions were given.

After the second injection, the pannus disappeared from the left cornea, leaving a very faint scar. The right cornea showed a marked recession of the pannus. After the third injection, a week after the second, there remained only the part on the right cornea, and after the fifth injection the pannus had entirely disappeared (November 11, 1913). At present (December 6, 1913), both corneas continue clear except for a scarcely perceptible scar in each. Streptococci were found in the cultures made from the conjunctival curettings. Curiously, her skin condition improved also, and now her skin is absolutely clear.

Case II.—A. M., 15 years old. "Sore eyes" 3 years. Operation (expression?) New York Eye and Ear Infirmary, February, 1912. Came to our clinic July 10, 1913, with a papillary conjunctivitis of both eyes, more pronounced in right, marked and very active pannus covering more than half right cornea and a small scar of a former ulcer on left cornea. The Von Pirquet test was negative. In spite of this, she was given in increasing weekly doses subcutaneously for seven weeks tubercle bacillus emulsion, beginning with 1/10000 mg. At the same time atropine, hot applications and yellow oxide of mercury ointment, 2½ per cent., in conjunctival sac were used daily. Bluestone was used at intervals. At the end of this time the very active symptoms had cleared up, but the pannus of upper half right cornea, persisted. During the last week of the T. B. E. injection, September 6, 1913, the old ulcer on the left cornea became active. The T. B. E. injections were stopped, and on September 16, 1913, an inoculation of 25 million streptococci (stock vaccine) was given. This was continued in doses increased by 25 millions once a week. After the third injections (in 16 days), the ulcer on left cornea was healed, leaving a very light scar which still persists (December 6th). The pannus of the right eye receded slowly, and after the sixth injection (October 22d), the infiltration of the cornea, from being moderately dense, had become very light, so that the iris could be clearly seen, and the blood vessels running in from the top had become reduced to only a few of the larger ones. On October 30th a mixture of streptococci (175 millions) and staphylococcus pyogenes aureus (500 millions) was injected. Two days later (November 1st) there was a striking improvement noted. There was left only a little infiltration of the cornea at the top, with one or two small vessels running into it. By November 11th the right cornea was almost normal, except for a slight rim of cloudiness at extreme top. At present (December 6, 1913), there is still this small hazy rim at the top. The thickening of the palpebral conjunctiva has decreased, so that the left palpebral conjunctiva is still reddened, but has become thin enough to allow the palpebral blood vessels to show to a certain degree.

Case III.—M. L., 33 years, married, came to the clinic December 21, 1911, with a cicatricial condition (non-operative) of both palpebral conjunctiva and sluggish pannus of both corneas. Duration of eye trouble, 4 years or more. She was treated in the usual way with hot applications, atropine, yellow oxide of mercury ointment and bluestone. She had continued under constant observation. March 3, 1912, she had an acute exacerbation with intense vascular reaction of pannus, first in right and

then in left eye. The right cleared up under the above treatment. While pannus was still acute in the left eye (July 27, 1912), patient was given 50 million polyvalent hemoglobinophilic bacilli. The injections in increasing dosage were given weekly for 9 weeks. By September 28, 1912 (2 months), both corneas had improved to such an extent that there was only a very light cloudiness of the upper one-fourth. On October 15th, while these injections were still being given, an active acute pannus started up in right cornea, and on November 9, 1912, in left. The injections and local treatment were continued, and in two weeks all active symptoms were gone, leaving the corneas slightly cloudy again at the top. This latter state lasted seven months, until June 5, 1913, when a mild exacerbation of pannus in left cornea appeared, lasting only a few days. Mrs. L. was at this time pregnant. August 21st her baby was born. Eight days later she appeared at the clinic with a very severe reaction in the left eye. There was a dense opacity of the whole cornea, marked ocular and ciliary injection. September 4, 1913, six days after onset, 25 million streptococci were injected. In five days improvement was noticeable; by the seventh day, on which second injection was given, the improvement was marked; by the ninth day the acute symptoms had subsided. After the fourth injection they were clear except for slight blurring above, and after the eighth injection (November 1st), both corneas became quite transparent with no show of pannus. Streptococci, staphylococcus pyogenes aureus and xerosis were found in cultures.

Case IV.—A. P., age 10 years; thin, pale and undernourished child. First seen March 4, 1912, with "Follicular Trachoma" and pannus in both eyes. Under daily local treatment and injections of hemaglobinophilic bacillus vaccine (begun October 15, 1912). After nine months (December, 1912), the pannus in left had become less, and after another month considerable improvement was noted in right eye. By May 8, 1913, however, the pannus had returned and covered upper half right cornea and about upper third of left with a small active ulcer on left also. The child was sent to our country camp for nine weeks. He gained five pounds, and his general condition was much better, but the pannus, although a little less dense, was present in both eyes. September 11, 1913, a combined vaccine, streptococcus (25 million) and staphylococcus pyogenes aureus (200 million) was injected. Streptococcus vaccine alone was then given for three doses (weekly) and again the combined. After the third injection of the latter (October 30, 1913), the pannus of the left eye was entirely healed, leaving a very light scar, and the pannus of right was much less. The last injection was given November 16th. On November 18th a few fine ulcers appeared on both corneas. On November 20th, Tuberculin B. E. 1/10,000 was given. On November 25th the corneas had cleared and were more transparent than they had been since he was under operation. Left cornea shows only small scar and right the merest rim of infiltration at the top. After the second injection of tuberculin the corneas looked in good condition, but after the fourth (December 9, 1913), pannus had returned to both eyes, now covering fully half of each cornea Streptococcus injections now will be substituted for the tuberculin.

Case V.—Mrs. M., 40 years, married. Duration of disease 8 years, combined excision of Heisrath 5 years ago, canthotomy last year. Came to clinic December 13, 1910, with pannus of both eyes and trichiasis. Under our observation, with local treatment, her pannus had receded and relapsed at varying intervals. She was given the hemaglobinophilic vaccine from May 2, 1912, to April 12, 1913, at first regularly, then rather irregularly. At first there seemed to be marked improvement, then later no effect. For a time she was under the care of another dispensary. On October 4, 1913, she returned with a very severe active vascular keratitis, involving whole right cornea and part of left. She was given empirically 25 millions streptococci. For three days after this injection she was even worse, then began to improve the day following the second injection. Eyes were not seen immediately after second injection, but patient said they were worse for several days, then began to improve. On November 4th, fourth injection was given, and on November 20th, fifth injection. On this date her right cornea was almost clear, there were no active symptoms and there was only a slight cloudiness of left cornea. On December 5th both corneas were clear except slight blurring at top, and there were no active symptoms.

Case VI.—M. R., 17 years old. Came to clinic with severe double pannus. Could not work. Had a combined excision of palpebral conjunctival and tarsal cartilages. Under daily local treatment and two injections of streptococcus vaccine, he improved so much he has not returned to clinic.

Specific Keratitis.—Y. K., 15 years old, double keratitis of several years standing; dense opacity of both cornea. Came to clinic December 12, 1912. Von Pirquet test negative. Wassermann positive *January* 4, 1913, and *September* 16, 1913. Has had two injections of salvarsan—*February* 8, 1913, and *May* 27, 1913, respectively. Wassermann still positive almost four months after second injection of salvarsan. Has been taking mixed treatment by mouth. June 30th sent to summer camp for nine weeks, when she improved considerably. In September her corneas presented a very little infiltration, she could read and do her school work. On October 18, 1913, 25,000,000 streptococci were injected. On the following day there was an intense ocular and corneal reaction almost like that following the salvarsan. It lasted four days and left an increased cloudiness in the cornea. The second injection of streptococci was given November 11, 1913 (almost three weeks after first), with no reaction. After the third injection, following a week's interval, both corneas became clearer than they have been since her first visit to us. Five injections in all have been given, and now there are transparent areas in corneas with small faintly blurred areas.

Tuberculous Keratitis.—Case I.—I. B., 10 years old. Came to clinic June 11, 1912, with dense mottled bluish gray infiltration of left cornea with injection, photophobia and discomfort. Palpebral conjunctivas normal. Under usual local treatment she showed slight improvement by July 2d. On July 16th a fresh ulcer with active symptoms appeared, and by July 26th there was a distinct ectasia of cornea. The child again im-

proved slightly under local treatment until August 20th, when she received a blow on the left eye and appeared with a complete dense infiltration with ocular and ciliary injection. October 8, 1912, the Von Pirquet test gave a moderate reaction. Three days after this test was made a fresh crop of small ulcers appeared in lower part of left cornea. On October 18, 1912, tubercle bacillus emulsion 1/10000 mg. was injected. This was given weekly, increasing each dose by 1/10000 mg., until January 10, 1913, when 1/1000 mg. was given. After the third injection the cornea had cleared sufficiently to show the pupil; by the 6th the cornea was transparent except for few tiny macules. By January 10, 1913, the cornea looked so well that the tuberculin was stopped. April 15, 1913, a small fresh ulcer with local injection appeared on the cornea and tuberculin bacillus emulsion was again begun at 1/10000 mg. One week later the ulcer was healed, leaving a tiny scar. Injections, however, were continued and the dosage carried to 1/100 mg. (September 19, 1913). By August 5, 1913, the cornea was quite clear, and the last record (December 5, 1913), shows that cornea is still transparent, except for a few very small, almost imperceptible, blurs which are seen only in close inspection. The patient has gained a good deal in weight and general condition since T. B. E. was started.

Case II.—E. H., 9 years old. "Sore eyes" for 8 months, entered clinic May 18, 1912. Eye condition similar to case I, cornea ectatic. Gave a very pronounced reaction to the Von Pirquet test both at site of vaccination and locally in eye. Within a few days after the Von Pirquet test was made, there was a noticeable improvement in eye before the injections were begun. T. B. E., 1/10000 mg., given May 28, 1913. By June 8th the ectasia of cornea had disappeared and opacity was much less dense. By June 27th the lower part of cornea alone was hazy with scattered more dense flecks. By November 6, 1912, there was only the slightest blur on cornea. Patient had begun to be irregular in attendance. January 7, 1913, two fresh ulcers developed, but left after a few days under tuberculin. The child was not seen then until March 6, 1913, when she reappeared with several small ulcers on upper part cornea. 1/10000 mg. T. B. E. given. By March 13, 1913, was improved, and given second dose of 2/10000 mg. Got well and remained away from clinic until May 10th, when appeared with tiny ulcer just above pupil. Otherwise her cornea was quite clear. She received an injection of tuberculin and has not been seen since.

Case III.—J. S., 13 years old. Came to clinic October 22, 1912. "Sore eyes" for 2 years. Had several small ulcers on corneas, both eyes. Had photophobia and blepharospasm. Von Pirquet on December 20, 1912, positive. Eyes showed same improvement after test as in case II. T. B. E. begun December 20, 1912. Increased gradually to 5/100 mg. by June 24, 1913. On January 14, 1913, both corneas were clear, no photophobia—no blepharospasm. On January 28, 1913, a fresh ulcer appeared, but cleared up rapidly. On March 11, 1913, another small ulcer broke out which healed rapidly. By April 4, 1913, both corneas were transparent. Last ulcer appeared May 25, 1913. On October 31st both corneas were clear and continue so. Only irregular injections were given after June,

none at all during summer. A few follicles have appeared on palpebral conjunctivas since under our care. They were probably stimulated by the local treatment of atropine, etc.

Case IV.—F. C., 10 years old. Came to clinic April 1, 1913, with a severe vascular keratitis covering whole right cornea with dense infiltration. Left cornea had old scars. Duration of eye trouble 2 years; child had a healed tuberculous spine; Von Pirquet test positive. Tubercle bacillus emulsion was begun April 11, 1913, 1/10000 mg. On April 15th a slight ectasia of right cornea was noted. By May 13, 1913, there was much improvement and no active symptoms (after four injections). By June 6, 1913, right cornea was transparent except for a few light macules. Last injection, 2/1000 mg., was given June 28, 1913. Both corneas were clear except for light blur over centres. The condition was the same September 2, 1913, when patient was last seen.

Case V.—C. L., 2½ years. Came to clinic July 31, 1913. Left eye showed a complete deep lilac colored infiltration of whole cornea, thickly vasculized and totally obscuring iris and pupil. Cornea looked as if it had ruptured and healed. Child had a tuberculous knee. Mother had died of tuberculosis. August 2, 1913, Von Pirquet positive. Tuberculin injections, beginning with 1/20000 mg., B. E., were started August 15, 1913. After the second injection the vessels disappeared from cornea and cornea took on a bluish-gray tinge. The process had gone too far to have any hope of saving the eye, but the child's general condition and knee condition have improved wonderfully. From a peevish, fretful, pale child, he is now happy and cheerful, with a good color, and gets about well with a brace. On December 2, 1913, he was getting 3/1000 mg. T. B. E.

Staphylococcus Pyogenes Aureus Inoculations—

Acute Conjunctivitis: S. T., 50 years old. Staphylococci pyogenes aureus found in cultures. Came to clinic September 18, 1913, with intense ocular injection, injection palpebral conjunctiva, with much discomfort, profuse watery and some muco-purulent secretion. At the end of two weeks local treatment, with silver nitrate solution, argyrol, boric acid solution given daily by nurse at clinic, there was practically no improvement. On October 2, 1913, 1,000 million staphylococci pyogenes aureus (stock vaccine) was injected. Two days later the eye was well.

Infected Meibomian Gland: E. W., 40 years old. Abscess of meibomian gland ruptured into conjunctival sac and set up an acute conjunctivitis. Patient was a teacher and anxious to get to her work. Case was seen on third day, argyrol and boric acid advised. On following day the eye appeared about the same, and 500 million staphylococci pyogenes aureus were injected into arm. The improvement on the following day was striking, and the second day after the injection the patient returned to her work.

About a dozen other cases of infected meibomian gland, some of which had ruptured and some not, have been treated similarly with immediate and permanent results.

About 27 cases of styes, many of them recurring ones, have been given staphylococcus injections, varying from one or two to six injections, with very satisfactory results.

B. MICROSCOPIC AND CULTURAL STUDIES OF TRACHOMA AND ALLIED CONDITIONS IN THE PUBLIC SCHOOL CHILDREN OF NEW YORK CITY

By Anna Wessels Williams, Minnie Augusta Wilson and Caroline R. Gurley, assisted by Olga Povitsky, Lucy Mishulow, Percy Crane and Ella Lipsky

Introduction

A report of the beginning of our work with details of earlier methods and findings has already been given.[56] Having soon reached the conclution mentioned in the review of the literature (p. 160), that, notwithstanding the voluminous publications on the subject of the bacteriology of conjunctivitis, only a few comprehensive bacteriologic studies have been reported; and at the same time being impressed from our early microscopic studies with the resemblance in morphology and staining reactions of the "trachoma or Prowazek inclusions" to dense groups of small bacteria, we determined to make, in connection with the clinical work, as exhaustive a cultural and microscopic study of our cases as was practicable with the facilities at hand and with our present knowledge of the characteristics of pathogenic microorganisms.

In order to have our cases near the laboratory, we started, in one of the attached buildings, the "trachoma clinic" mentioned in the preceding papers.

Technic. When we began our cultural studies each case showing any inflammation of the conjunctiva, after being clinically examined and recorded as given on p. 172, was subjected to the following microscopic and cultural examinations. A light, but thorough curettage was made with an eye curette, usually over the upper tarsal and cul de sac regions. The curetted material was used chiefly for the films, but occasionally some of it was cultured. The films were made by cover glass pressure, were fixed immediately in methyl alcohol, C. P., and were stained over night in Giensa's solution. (See Gurley and Chase[17] for details of method of staining). Other stains—Gram's methylene blue, fuchsin and methylene blue mixture, etc.—were used from time to time.

The majority of the cultures were made as follows: Over the freshly curetted conjunctival surface was rubbed a sterile cotton swab which was placed immediately into a 10X 1 cm. tube containing a mixture of veal-broth and rabbit or horse blood 10:1, about 3 cm. deep. The tube was then sent to the laboratory, where further cultures were made. This preliminary blood-broth culture acts in two ways; first, immediately, as a diluent in cases where many organisms are present, and second, after growth in the incubator, as an enricher, especially for the group of hemoglobinophilic bacilli and for the gonococcus. From this blood-broth tube stroke cultures were made on plain veal agar, ascitics—glucose-ascitic—and blood (rabbit or horse)—veal agar and in deep tubes of these

media containing agar, inoculated while fluid, allowed to harden and then covered with more melted agar and, when solid, with albolene. Cultures, including the original tube of blood-broth, were kept at 36 degrees C. and examined in from eighteen to twenty-four hours, when a second series of cultures was made from the original blood-broth culture, diluting when necessary. Fishings and films were made from plates, and all cultures were returned to the incubator. In forty-eight hours fishings and films were made again; and all cultures were studied for varying times. The deep tube cultures were observed for anaerobes and were examined in from four to ten days. These deep tube cultures were discontinued after a short time, since we found no organisms growing in the depths that did not grow in the blood-broth tubes.

After we had examined in this way a number of conjunctivitis cases, we concentrated in cultural work on those diagnosed clinically as "active trachoma," as suspicious and as doubtful cases ("beginning trachoma"). From time to time through over full clinics a number of these cases did not get this complete examination, but such cases were recorded and examined later if they continued to be of interest. Only a few follicculosis cases were thus examined, and many of the acute catarrhal conjunctivitis cases cleared up too quickly for a later culture study.

In a number of cases, before cultural studies were begun, when we were interested chiefly in the morphologic examination of the "trachoma, or Prowazek, inclusions" we studied only smears; and in a number of cases later, especially of acute contagious conjunctivitis, where our chief point for a time was to determine the prevalence of the group of hemoglobinophilic bacilli as well as the relation of our hemoglobinophilic strains to the influenza bacillus and the Koch-Weeks bacillus, we studied only cultures.

We have then 812 cases examined by cultures, 500 cases examined by direct smears (chiefly for "Prowazek inclusions"), and 400 cases examined by both cultures and direct smears.

The 812 cases examined by cultures were obtained from the following places:

Cases from New York City school children and their families—	
Borough of Manhattan (lower east side)	607
Borough of The Bronx (upper east side)	38
Borough of Brooklyn	9
Cases from New York City Eye and Ear Hospitals	21
Cases from Blackwells Island	19
Cases from Ellis Island	16
Cases from Home for Feeble-minded	79
Cases from Kentucky mountains	13
Miscellaneous	10
	812

We see by glancing at Table I. that the hemoglobinophilic bacilli were found most frequently (excepting staphylococci, which were present in practically all conditions in varying numbers) in these cases, particularly in those diagnosed papillary conjunctivitis (at first called "acute trachoma") and acute conjunctivitis. The first strains of this group that we isolated from cases diagnosed clinically as "trachoma" (papillary conjunctivitis) were so extremely small and coccus-like that we thought we were dealing with a new variety; but we soon found that the different strains varied somewhat in size, and that the same strain in subsequent culture generations varied in the same particular. Then when we found that all the strains isolated from our acute conjunctivitis cases (including all cases of clinically typical "pink eye"), as well as from our subacute and chronic conjunctivitis cases, were strictly hemoglobinophilic, we came to the conclusion that these bacilli must be classed, at least provisionally, as influenza bacilli, and much of our subsequent study was directed towards determining whether these strains might be divided into distinct species or varieties of the influenza group or whether they all belonged to the one species, the *B. influenzae.*

We recognized further that from a clinical standpoint, too, it was important to determine the relationship of our strains to the Koch-Weeks bacillus, since so many of our acute cases were clinically typical "pink eye," or "acute contagious conjunctivitis" and since several of our early cases passed from this condition to that of papillary conjunctivitis showing "trachoma inclusions." So we set about systematically hunting for the classic Koch-Weeks bacillus. For this purpose we chose a series of early marked typical "pink eye" cases before treatment and submitted them to our miscroscopic and cultural technic. In all of these we obtained in our original tubes and plates abundant and practically pure growths of a strictly hemoglobinophilic bacillus similar to our other strains. We fished from many different colonies in each plate, but no classic Koch-Weeks bacillus was found. The original smears showed bacilli sometimes larger (the Koch-Weeks bacillus?), sometimes shorter (the influenza bacillus?), but only abundant hemoglobinophilic bacilli grew. That we may have missed a bacillus answering to the classic Koch-Weeks bacillus is possible. One has only to remember the history of the demonstration of the Bordet-Gengou bacillus to realize how unwise it is to draw hasty conclusions from resemblances between morphology in cultures and that in direct smears. In these pertussis studies at first only hemoglobinophilic bacilli were isolated from pertussis cases. Finally the distinct variety, *B. Bordet-Gengou,* was grown. It may be that we, in the same way, have not yet been able to grow the classic Koch-Weeks bacillus. While we do not believe this, all we can positively say is that in this large series of cases of clinically typical "pink eye" we have gotten in direct cultures abundant growths of a strictly hemoglobinophilic bacillus, which answers to the description of the influenza bacillus rather than to that of the classic Koch-Weeks bacillus. And this fact has led us to agree with those who consider the Koch-Weeks bacillus a strict hemoglobinophile, indistinguishable so far from the *B. influenzae.*

TABLE I

THE FOLLOWING TABLE SHOWS THE NUMBER OF CULTURED CASES GROUPED ACCORDING TO CLINICAL DIAGNOSIS AND CULTURAL FINDINGS

Diagnoses	Total No. Cases	Hemoglobinophilic B.	Pneumostreptococcus group	Strept. Pyog. (Hemolyzing)	Gonococcus	Morax Axenfeld	Tetragenus	Yeast	Diphtheria	Typhoid colon group	Staphlococcus Pyog.	Xerosis B.	No growth	Micro. Catarrhal
"Old Trachoma"	59	9	14	..	..	1	..	..	..	1	35	25	..	..
Papillary Conjunctivitis	237	147	60	2	5	6	2	5	..	4	190	95	1	..
Follicular Conjunctivitis	145	49	6	1	..	8	..	..	..	4	130	65	..	..
Acute Conjunctivitis	271	204	95	4	8	2	..	..	2	5	250	156	2	3
Folliculosis	45	..	..	..	..	..	..	..	..	..	19	15	23	..
Other Conditions	55	15	18	..	..	..	..	..	..	3	32	31	..	..
Total	812	424	193	7	13	17	2	5	2	17	656	387	26	3

The following tests were made in order to help determine the characteristics of our strains.

I. *Hemoglobinophilic Tests.* We went very minutely into the question of the hemoglobinophilic property of our strains. Most of the experiments relating to this point were made by Miss Mishulow. Besides the preliminary attemps to cultivate these strains on blood-free media, we made repeated attempts from time to time on a large number of the older strains, all with negative results. We tried to accustom the strains to growing without blood by growing them in gradually diminishing amounts of it. We found that though they grow well in amounts up to 1-1000 (horse blood) the growth stops after a variable number of culture generations when this amount is lessened. Horse hemin crystals* in veal agar or broth, in dilutions of 1 to 100 and 1 to 1,000, gave no growth after the third culture generation.

Horse serum, obtained by drawing the blood in sodium citrate and, after allowing the red blood corpuscles to settle, pipetting off the clear supernatant fluid, gives variable results. Some serums allow growth for many generations and others for only a few. In the former case it cannot be denied that the positive result is probably due to the presence of a minute amount of hemoglobin. When we remember that in 1 to 1,000 blood dilution it is only detected by a more delicate test than the spectroscope (*e. g.,* Adler's benzidene test), we may realize how easily, by the method used for obtaining the serum, hemoglobin in amounts large enough to allow growth might be present and might escape detection. The positive cultures claimed for the Koch-Weeks bacillus in media containing "rich hydrocele or ascitic fluid" may be explained by the presence of such a minute amount of hemoglobin.

Symbiosis Tests. In connection with the attempts to makes these strains grow without hemoglobin, we carried on a number of so-called "symbiosis" tests, *i. e.,* we grew certain strains on blood-free agar and broth coincidently with other bacteria. The results obtained on blood-free veal agar are condensed in Table II. All these tests were repeated with practically the same results. In blood-free bouillon strains 636 and 351 (hemoglobinophiles) grew well with xerosis bacillus for 12 culture generations. BI. 1 (influenza bacillus) was negative on the sixth generation in the first test, but had a growth on the eighth in the second; while BI. 2 (influenza bacillus) failed to grow beyond the seventh culture generation in this fluid medium. As plain and blood-veal agar and broth were used as controls in this work we had a further test of the hemoglobinophilic property of these strains. Many individual tests were made of symbiosis on plates as well as in tubes, which bore out the results shown in Table II., namely, that all the organisms tried, except *B. xorosis, B. diphtheriae* and the gonococcus, interferred with the growth of these strains. This is entirely contrary to all statements in regard to the influence of the staphylococcus on this group of organisms, and we cannot explain the apparent contradiction. It is true that on plates, if the staphylococci are

* Isolated by Mr. Atkinson.

TABLE II

GROWTH OF HÆMOGLOBINOPHILIC BACILLI IN SYMBIOSIS ON BLOOD-FREE VEAL-AGAR

STRAINS OF HÆMOGLOBINOLHILIC BACILLI	SPA		SPA+STREP.		XEROSIS		XER+STREP.		STREP.		GONOCOCCUS		DIPHTHERIA		B. HOFFMANNI	
	Gen.	Growth	Gen.	Growth	Gen.	Growth	Gen.	Growth	Gen.	Growth	Gen.	Growth	Gen.	Growth	Gen.	Growth
636 (from Acute Trachoma).....	1	—	1	—	20	+	3	—	4	—	19	++	12	+	2	—
351 (from Acute Trachoma).....	..	..	..	..	20	++	2	—	2	—	20	++	12	±	5	—
BI. 1 (from Meningitis).....	1	—	1	—	5	—	3	—	4	—	20	+	11	±	4	—
BI. 2 (from Pneumonia).....	..	..	..	..	14	++	..	..	..	..	..	..	12	±?	2	—

few, isolated colonies of the hemoglobinophilic bacilli on the first culture generation from a blood-agar medium or on blood-agar media may be larger in the neighborhood of staphylococcus colonies, but only incidentally so, we believe. Attempts at symbiosis growths with cultures of staphylococci, xerosis bacilli or diphtheria bacilli killed at 60 degrees C. the first two for one and a half hours and the last one for fifteen minutes, were negative after three to seven culture generations. The practical point gained from this study is that when many staphylococci are present coincidentally with hemoglobinophilic bacilli in a case of conjunctivitis, the former may interfere with the isolation of pure cultures of the latter. In order to try to inhibit the growth of the staphylococci in mixed cultures and allow the growth of hemoglobinophilic bacilli, Churchman's gentian-violet test was made by Miss Wilson and Miss Mishulow. Details of these tests are as follows: Dilutions, ranging from 1-50 to 1-1,000, of a saturated aqueous gentian-violet solution were made in sterile blood-broth. These dilutions were distributed into small test tubes, and inoculated with practically equal quantities of 24-hour agar cultures of strain 636 (*hemoglobinophile*), *staphylococcus pyogenes aureus and xerosis* bacilli in pure culture and in mixtures. Controls of each were made in blood broth. Blood streaked plates were made of these cultures immediately after inoculation and also after ½, 1, 2½, 24 and 48 hours' incubation at 36 degrees C. These plates were examined after 24 hours, 48 hours and three days incubation. On the whole the best results were obtained after one hour's incubation in the Gentian-violet-blood-broth.

TABLE III

One (1) Hour Gentian-Violet Broth Emulsion

Culture	636+SPA+Xer			636+SPA		636+Xer		636	SPA
Control	±	+++	+	—	++	+	+	+	++
G.V. dil. 1– 50	±	—	—	—	—	±	±	—	—
" 1– 100	±	—	+	±	+	±	±	±	±
" 1– 200	±	—	±	—	+	±	±	±	+
" 1– 400	+	+	+	+	+	+	+	+	±
" 1– 500	+	±	±	+	+	+	+	+	+
" 1– 600	+	+	+	+	+	+	+	+	+
" 1– 800	+	+	±	+	±	+	+	+	+
" 1– 900	+	±	+	+	+	+	+	+	+
" 1–1000	+	+	±	+	+	+	+	+	+
3 - day gentian - violet plate all dilutions	—	—	—	—	—	—	—	—	—

The results, as indicated in Table III., show that, although the growth of the gram-positive organisms was not completely inhibited in the mix-

tures which gave a good growth of the hemoglobinophilic bacilli, it was restricted to a considerable extent. Thus in the controls of the mixed cultures the plates had a heavy growth of staphylococcus pyogenes aureus after 24 hours' incubation and were completely overgrown after 48 hours; whereas, on the plates of the gentian-violet cultures, even when there was a good growth of staphylococcus pyogenes aureus, it was not heavy and the colonies were discrete. Two plates of the mixed cultures of strain 636 and staphylococcus pyogenes aureus on the 1-50 dilution, one made immediately after inoculation, and the other after one hour incubation, had a pure growth of hemoglobinophilic bacilli in the centre and a good growth of staphylococcus pyogenes aureus on the magrin of the blood zone. Also several plates of a pure culture of staphylococcus pyogenes aureus on 1-50, 1-100 and 1-200 dilutions showed a similar growth. Evidently the growth on staphylococcus pyogenes aureus was inhibited in the centre of the plate by the presence of a small quantity of gentian-violet carried to the plate with the culture; since the loopful of material is placed in the centre and then radiated from the centre.

The same dilutions of gentian-violet used in the blood-broth tests were made in liquid agar at a temperature of 55 degrees C. and plates poured immediately. When they were solidified a drop of rabbit's blood was put in the centre of each plate. Cultures of hemoglobinophilic bacilli 636), staphylococcus pyogenes aureus, xerosis, in mixtures and alone, as before, were inoculated into blood broth and from these the gentian-violet and control plates were made. The growth on the gentian-violet plates was negative on all dilutions, while the control plates showed growth as indicated in Table III.

We found by this study that by using the first method described, *i. e.*, inoculating a blood-broth-gentian-violet medium with material containing hemoglobinophilic bacilli in mixed culture with staphylococci, that we might expect material aid in isolating pure cultures of the former. Unless all aids possible are used in a cultural study of these cases, we feel that a certain percentage of infections with hemoglobinophilic bacilli, as well as other micro-organisms, may be missed.

Virulence Tests.—In connection with the study of antibody production by Dr. Povitsky (p. 93) a comparatively few tests were made of the degree of virulence possessed by our strains for rabbits and guinea pigs. The results indicate on the whole a similarity to strains of hemoglobinophilic bacilli isolated from other parts of the body. That is, recently isolated strains are irregularly virulent for rabbits and guinea pigs on intraperitoneal inoculation, and the virulence which is rapidly lost on artificial cultivation, may be markedly increased for a species (guinea pigs) by successive animal passages.

Eight monkeys, four macacus species, and four dog-faced baboons were inoculated into the conjunctiva with 48-hour cultures of eight different strains. One out of the eight (a macacus) showed, after repeated inoculations, a subacute conjunctivitis, during the course of which a few superficial follicles developed. No typical "trachoma inclusions" were found, though some "transition forms" were seen.

Agglutination Tests (see p. 93).—A strain of hemoglobinophilic bacilli from an Ellis Island case (a deportable "trachoma" case from Poland) produced agglutinins of 1 in 2,400 for itself and one from our public school "trachoma" cases, but not for two other public school strains or the strain from influenzal meningitis. Other strains, however, from public school cases produced agglutinins for this meningitis strain.

The only inference we can draw from these studies is the same as that drawn from studies on agglutination in hemoglobinophilic bacilli from other source; namely, that in the hemoglobinophilic group of bacilli there are distinct varieties as regards agglutinin productions, but that these varieties seem not to be related to any definite set of morphologic characteristics or clinical symptoms.

The complement binding test has not yet given satisfactory results. This is chiefly because no antigen has been obtained that would show a specific fixation, apparently because of the serum content of the culture media used. Recently, by growing organisms on media containing a minimum amount of heated blood, *i. e.,* coagulated blood-agar (1 part of blood in 500 parts of agar, at 90 degrees C.), we are apparently beginning to make good antigens. It may be that with further testing, the fixation of complement phenomenon may help in a study of these strains.

During the attempts to obtain a good antigen, we have tried various methods of obtaining abundant growths of our strains in a medium containing no substance that might interfere with good antigen production. Thus we again tried to lessen the serum content of our medium by gradually diminishing the amount of blood used, but we found, as before, that beyond 1 to 500 dilution we were apt to get scant growths, and beyond 1 to 1,000 no growth. Even in the dilution of 1 to 500 the growth is not always abundant unless the blood is added to very hot agar. 90 degrees C. seems to be sufficient to destroy something in the blood that is deleterious to growth, for, as I have said, Dr. Povitsky has found that growth is very luxuriant on media made in this way. On salt-free blood-agar growth is as abundant as on the mixture of ordinary veal-agar and blood.

So far, however, neither the complement binding test nor any other test tried has helped us differentiate our hemoglobinophilic strains from the *B. Influenzae,* or the Koch-Weeks bacillus. And until we can make this differentiation we must consider all of these organisms as possibly identical in potential pathogenic power; just as we consider a pneumococcus isolated from the eye similar in possible pathogenicity to a pneumococcus from any other source.

Relation of Hemoglobinophilic Bacilli to "Trachoma Inclusion."—In studying closely the morphology in cultures of these bacilli, we were struck by the fact that they frequently grow in more or less dense clumps of extremely minute and irregular coccoid forms. This led us to the conclusion that possibly they formed the "trachoma, or Prowazek, inclusions" and when we found that these bacilli and the inclusions were found coincidently and repeatedly in so many cases diagnosed as acute papillary trachoma (see Table IV.) the possibility became a probability. So we proceeded to study the morphology of the cultures more minutely. Each

TABLE IV

COINCIDENCE OF HEMOGLOBINOPHILIC BACILLI AND "TRACHOMA INCLUSIONS" IN CASES EXAMINED BOTH BY DIRECT SMEARS AND BY CULTURES

Diagnosis	"Trachoma Inclusions"		Hemoglobinophilic Bacilli		Remarks
	+	−	+	−	
"Old Trachoma"	9	12	7	14	Seldom any growth on original plates. Growth in enriching media."
Papillary Conjunctivitis.....	184	53	177	90	In many of the recovering cases bacilli and "inclusions" are found irregularly.
Follicular Conjunctivitis.....	2	22	14	10	
Acute Catarrhal Conjunctivitis[1]	17	71	45	43	Among these cases are included 38 cases of gonorrhoeal ophthalmia, 9 of which showed "trachoma inclusions" and recovered in from 3 weeks to 2 months. The others were cases of acute catarrh accompanied by hemoglobinophilic bacilli, which recovered in average of 6 weeks.
Folliculosis.....	0	18	0	18	"No carriers" found.
Other conditions .	0	12	4	8	Most of these cases were blepharitis and keratitis accompanied usually by slight catarrh. Only examined once in this way.

[1] A number of cases (20) diagnosed first as acute contagious conjunctivitis developed later papillary conjunctivitis. These are included here among the papillary conjunctivitis cases.

culture was kept under observation for many days. Each day spreads were made from the same culture and stained by Giemsa's method exactly as we stain our direct films for the detection of the "trachoma inclusions." Other stains were also used, but Giemsa's was found to be most characteristic. With Gram's method the inclusions are decolonized. In examining these smears we found cultures of these bacilli, as they grow older, alter significantly both in their morphology and in their staining reactions to Giemsa. Twenty-four hour cultures show dense clumps of short bacilloid or coccoid forms taking a comparatively deep blue stain exactly as do the early granules of the "trachoma inclusions" (the "initial bodies of Lindner"). The morphology varies somewhat with the age of the inoculated culture, the date from isolation, the kind of medium and the strain. In forty-eight hours the forms become somewhat more irregular. Then in three days most of the bacilli have become extremely minute, many showing only as reddish granules (the "elementary bodies of Prowazek"), while scattered through the culture are swollen spheroidal bodies taking a fainter clear blue stain (the larger "initial bodies of Prowazek"), in some of which are minute reddish granules (more of the "elementary bodies of Prowazek"). A number of irregular light blue bodies are also scattered through the culture. Where the bacteria are densely grouped more red granules may appear in the centre of the group then at the periphery and more blue bodies at the

periphery than in the centre. In short, all of the changes described by Prowazek and others as characteristic of "trachoma inclusions" are seen in the growing cultures of these hemoglobinophilic bacilli (plate I, figs. 1 to 6, inclusive).

Similar day to day studies were undertaken with the other types of bacteria found most frequently in the eyes diagnosed as trachoma, *e. g.*, streptococci, staphylococci, gonococci, a minute gram-negative non-hemoglobinophilic bacillus not before described found in a few cases of papillary conjunctivitis, xerosis bacillus, and micrococcus tetragenus, but in none of these varieties except the gonococcus were similar changes found in the same marked degree. Cultures of the tetragenus coccus, isolated repeatedly from a case of chronic conjunctivitis, showed some dense bunches but practically no red granules. This corresponded with what was found in direct smears from the case. Many of the epithelial cells contained the tetrades, some distinct and some in dense clumps, approaching in morphology the "early trachoma inclusions" found in ophthalmia neanotorum, but no forms containing reddish granules were seen.

Relation of Gonococci to "Trachoma Inclusions."—The fact that the gonococcus cultures showed so definitely appearances similar to the "trachoma inclusions" led us to make a special study of a series of ophthalmia neanotorum cases, thirty-eight in all. The cultural technic employed was the same as that used for the other cases and we were on the lookout particularly for gonococci and hemoglobinophilic bacilli. In most of these thirty-eight cases treatment had already been begun when our cultures and films were made, in fact, many of the cases were already near recovery, so we were not able to get a complete cultural and morphologic picture; however, the results as given in Table V. are rather suggestive. Gonococci were demonstrated by us in both smears and cultures in ten cases, in smears alone in nine cases, in cultures alone in three cases, and in neither culture nor smear in sixteen cases. Most of the cases giving negative results and some of the others were examined several times. This picture of irregular results is one that might be expected from recovering cases. From only three of these cases were hemoglobinophilic bacilli isolated. In nine cases definite "trachoma inclusions" were seen, in only one of which hemoglobinophilic bacilli were found as well, while in seven gonococci were found. In two showing "trachoma inclusions" no organisms were demonstrated in either smear or culture. A minute study of the direct smears from these eyes apparently throws more light on the subject. In all of the "inclusion" cases where gonococci are found apparent transition forms between gonococcus and inclusion are very evident, and we find from a further microscopic study of these slides that the inclusions on the whole present certain characteristics different from those found in our series from school children, produced, according to our hypothesis, by nests of growing hemoglobinophilic bacilli. The "inclusions," produced, as we think, by the gonococci are characterized (1) in early stages by being more definitely coccus-like at the periphery of the clump and more densely clumped in the centre; (2) in later stages by showing on the whole smaller

TABLE V

COINCIDENCE OF "TRACHOMA INCLUSIONS," GONOCOCCI AND HEMOGLOBINOPHILIC BACILLI IN CASES OF OPHTHALMIA NEONATORIUM

Number of Cases	Smears			Cultures		Cases Terminated (in Weeks)
	Trachoma Inclusions	Transition Forms	Gonococci	Gonococci	Hemoglobinophilic Bacilli	
No. 1	—	—	—	—	—	4
No. 2	—	—	—	—	—	4
No. 3	—	+	+	—	—	5*
No. 4	+	+	—	+	—	5*
No. 5	—	—	—	—	—	5*
No. 6	+	+	—	+	—	8*
No. 7	—	+	+	—	—	8
No. 8	+	+	+	—	—	3*
M 1	—	—	—	—	—	4
M 2	—	—	—	—	—	2
M 3	+	+	+	—	—	3*
M 4	—	—	—	—	—	2
M 5	—	—	—	—	—	5*
M 6	—	—	—	—	+	6*
M 7	—	—	—	—	—	5*
M 8	—	—	—	—	—	4*
M 9	+	—	—	—	—	5*
M 11	—	—	—	—	—	2
M 12	—	—	+	+	—	4*
M 13	+	—	—	—	—	4*
M 14	+	+	+	—	—	4*
M 16	—	+	+	+	—	7
M 18	—	—	+	—	—	2 (died)
M 19	—	—	—	+	—	3 "
M 20	—	+	+	—	—	4
M 21	—	+	+	+	—	4
B 136	—	—	+	+	—	8
1682	+	+	+	+	—	7
1686	—	—	+	+	—	6*
1687	—	—	—	—	—	2
1707	—	—	—	—	—	2
1708	—	—	+	—	+	3
1709	—	—	+	+	—	3*
1710	—	—	—	—	—	2
1714	—	—	+	—	—	8
1715	—	—	+	+	—	3
1716	—	+	+	+	—	6
1720	+	+	+	+	+	4

* Reported well when left hospital just before our last visit.

red granules than those produced by the hemoglobinophilic bacilli. These same differences are observed in a comparative day to day study of pure cultures of these two species of bacteria. (Compare figs. 7 to 12, inclusive, in plate). We have found different strains of each species vary in size of granules produced, so the second characteristic is not a clean-cut one.

Conclusions.—From this comparative morphologic study of "inclusions" and cultures we have reached the following conclusions:

1. The well-known power of the epithelial cells of the conjunctiva as well as epithelial cells of other parts of the body to harbor foreign invaders (*e. g.,* erythrocytes, leucocytes, several species of micro-organisms, notably gonococci and hemoglobinophilic bacilli) may be in evidence in any stage of inflammation.

2. Under certain conditions of infection, chief of which are, probably, a lessened virulence on the part of the invading organism and a lessened susceptibility on the part of the invaded cells, or one or the other of these conditions, some invaders (notably gonococci and hemoglobinophilic bacilli) may grow to a varying extent within the cells in groups or nests and thus produce the characteristic changes described as "trachoma inclusions."

3. Under these conditions, too, growth of the invader may not be limited to the epithelium alone but may extend to the deeper tissues. According to the extent and kind of irritation produced by such growth, a variable degree of proliferating inflammation of the surrounding tissue may be established, which later, if unchecked, may go on to the formation of fibroid tissue.

4. If it be true that both gonococci and hemoglobinophilic bacilli (and possibly other species of bacteria) may grow in epithelial cells in nests presenting a morphologic cycle similar to that described for the "trachoma inclusions," it explains, as one of us (Williams) has pointed out[56] the contradictory findings reported in cases of "inclusion ophthalmia," gonorrhoeal conjunctivitis and *inclusion inflammation* in the mucous membranes of urethra and vagina ("trachoma of the urethra or vagina") and of other parts of the body. That is, in a large number of cases of "papillary conjunctivitis" and a certain number of cases of ophthalmia neonatorum, as well as in a certain number of cases of inflammation of the mucous membranes of other parts of the body (*e. g.,* vagina, urethra), the "trachoma inclusions" found are due to one or more varieties of hemoglobinophilic bacilli; in a certain number of gonorrhoeal ophthalmia as well as in gonorrhoeal inflammation of the mucous membranes of other parts of body, the "trachoma inclusions" are due to the gonococcus.

According to one of the later reports of Leber and Prowazek[24] and the recent report of Noguchi and Cohen[42], certain "inclusion conjunctivitis" cases may be caused by other micro-organisms than the two mentioned above.

Other Micro-organisms Found.—Since several varieties of organisms other than the hemoglobinophilic bacilli and the gonococci are frequently present in cases diagnosed clinically as trachoma, the question of their action may now be discussed. Of course, from practically all cases with any secretion more than one variety of micro-organisms may be grown. Particularly is this true when a fluid medium is used for an original culture. Just how far enriching fluid media may be relied upon to aid us in determining the pathogenicity for the host of the organisms isolated

by this method has not yet been accurately determined. We know, however, that under certain conditions, organisms exerting an active pathogenic action in the body of the host are difficult to isolate and to grow immediately after isolation on artificial culture media. An enriching medium under these circumstances may be the only means to demonstrate the most harmful organism present. But, without doubt, it cannot give us a correct idea of the number of organisms present in the lesion at the time the culture was taken. We must have in mind these and other questions already considered when we try to interpret all of the bacteriologic findings in these studies.

Staphylococci.—We see by Table I. that the staphylococcus pyogenes group was demonstrated in nearly all of the cases. But in only comparatively few of these did a large number of colonies grow on the original plates, and these cases presented no uniform or related clinical picture. Original plates from the great majority of the cases showed few or no staphylococcus colonies. In the latter case the enriching medium used showed them after twenty-four hours.

The well-known power of the group of staphylococcus pyogenes to vary in pathogenicity coupled with the effect of the varying degrees of susceptibility or immunity of the host, could produce, as many have pointed out (see Duane & Hastings[12]) many grades of pathogenic action. To determine the exact conditions and limits of pathogenicity of this group would require much more work than we have been able to give in this study. We do know, however, that this group of organisms has been found in a number of cases of ulcer of the cornea, and in several cases of acute follicular conjunctivitis, acute exacerbations of "trachoma" and acute pannus as apparently the predominating organism present (judging from growth on original plates) in the curetted conjunctiva.

It seems, then, that in certain cases these cocci may play a most important part, not only in certain definite infections, but also in helping along a case of follicular conjunctivitis to become a "bad case of trachoma."

Streptococci.—We see, by reference again to Table I., that many streptococci were found. These were all of the short chained, non-hemolyzing type. In order to help determine the relationship of these strains to the great group of pneumo-streptococci, twenty strains isolated respectively from twenty successive cases showing these streptococci in original cultures, were studied both for their hemolyzing and their fermentative powers. Table VI. shows the condensed results of this study.

We see by studying this table that 45 per cent. of these strains are typical pneumococci, while 55 per cent. may be classed as *Streptococcus viridens* (producing in blood-agar plates a green halo about each colony instead of a zone of hemolysis), or, if Gorden's classification is used, we would have the latter divided into *Streptococcus pyogenes* 15 per cent., *Streptococcus salivarius* 20 per cent., and streptococci unclassed 20 per cent.

The fact that so many of these streptococci are of the type that is supposed to produce chronic inflammations in other parts of the body (e. g., in chronic articular rheumatism, endocarditis, etc.) is very significant. They seem to be more frequent in the subacute and chronic cases. In the pannus cases studied, where they are very frequent, the part played by these streptococci may be of great importance. This point is still under study. We must remember that members of this group of organisms show also a variable pathogenic power, and that the host responds with a variable degree of susceptibility or immunity.

The other species of micro-organisms noted in Table I. were found too infrequently to be considered as more than incidental aids in presenting an individual picture of a stage in chronic conjunctivitis which might be considered clinically as "trachoma in exacerbation." Thus there were several of these cases, notably the tetragnus and the yeast infections, that passed through a clinical course of conjunctivitis lasting several months, and were diagnosed for some time as papillary trachoma with follicles. These eyes finally became completely normal. They were all through most of their course mixed infections; one of the yeast cases had also the hemoglobinophilic bacilli, the other two yeasts infections and the two tetragnus infections showed streptococci as well.

Anaerobic Cultures.—When the cultural work began, deep tubes of the different media used were inoculated and grown under albolene. We discovered the interesting fact that under these conditions both hemoglobinophilic bacilli and gonococci would grow in the depths of the tubes. When we found that no unidentified organisms grew in this way in a series of cases diagnosed then as trachoma and which contained "trachoma inclusions," this method of growth was discontinued. That we may have missed important organisms by discontinuing this method is possible. We have not worked out all of the organisms found in the tubes of enriching media. We have diagnosed them from their morphology and staining characteristics as belonging to one or the other of the groups mentioned in Table I. In a few cases of papillary conjunctivitis we have found a minute gram-negative bacillus which grew abundantly on all media. In a few other cases in the same condition, we found a minute gram amphiole bacillus which we did not isolate. And, no doubt, a number of cocci escaped us, but none of these were frequent enough either in the original plates or in the enriching media to be considered of pathogenic importance.

Summary and Conclusions.—In summing up this cultural work, we wish to emphasize the following points:

1. Hemoglobinophilic bacilli are present much more frequently than was thought in cases of chronic as well as of acute conjunctivitis.

2. These bacilli were obtained in abundant cultures from all cases examined that were diagnosed clinically as "pink eye" or acute contagious conjunctivitis. No classic Koch-Week's bacillus was found; therefore the bacillus apparently producing "acute contagious conjunctivitis" in these cases is a strict hemoglobinophile.

3. None of the 424 strains of hemoglobinophilic bacilli isolated in this series can be so far differentiated from the influenza bacillus.

TABLE VI.

Fermentation and Haemolysis Tests of Pneumo-Streptococcus Group.

Culture	Milk			Neutral Red			Sacch.		Lactose		Raffin		Inulin			Salicin		Mannite		Haemolysis	
	Test	Clot	Acid	Test	Reaction	Gas	Test	Acid	Test	Acid	Test	Acid	Test	Acid	Clot	Test	Acid	Test	Acid	Green Producer	Haemolysis
21-79	3	+	+	2	–	–	1	+	1	+	1	+	4	+		2	+	3	–	+	+
H 1658	3	+	+	2	+	–	1	+	1	+	1	++	4	+		3	+	3	–	+	–
545	2	+	+	1	–	–	1	++	1	++	1	+	2	+		3	+	3	–?	+	–
138	2	+	+	1	+	–	1	++	1	++	1	++	4	+		2	+	3	+	++	–
150	2	–	+	1	+	–	1	++	1	++	1	+	3	?		2	+	3	–?	++	–
P S 371	2	+	+	1	+	–	1	+	1	++	1	++	3	+		2	+	2	–	++	–
H 2225	2	+	+	1	–	–	1	+	1	++	1	++	3	+		2	+	2	–	++	–
P S 108	2	+	+	1	–	–	1	++	1	+	1	++	2	+		2	+	2	+	++	–
21 391	1	+	+	1	+	–	1	++	1	++	1	++	1	+		1	++	1	+	+	–
Ros 375	3	+	+	2	+	–	1	+	1	+	1	–	3	–		3	–?	3	–	++	–
2185 A	2	+	+	1	+	–	1	+	1	+	1	+	4	–		2	–	3	–	+	–
562	2	+	+	1	+	–	1	++	1	++	1	–	4	1		2	–	3	–	++	–
1035	1	+	+	1	++	–	1	++	1	++	1	++	1	–		1	–	1	–	+	–
M 19 Re	3	+?	+	2	–	–	1	+	1	+	2	?	4	–		3	?	3	–	+	–
G 520	2	+	+	1	–	–	1	+	1	++	1	–	3	–		2	?	3	?	+	+
H 2224	2	–	+	1	–	–	1	+	1	+	2	–	2	–		2	?	2	?	++	–
311 A	2	+	+	1	+	–	1	+	1	++	1	±	2	–		2	++	2	+	+	–
21-190	3	±	+	2	+	–	1	+	1	+	1	+	4	–		3	+	3	–	+	–
2253	1	+	+	1	+	–	1	+	1	+	1	+	1	–		1	++	1	–	+	–
G 174	1	–	+	1	+	–	1	+	1	+	1	–	1	–		1	+	1	+	–	+

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4. These bacilli are found in cases of papillary conjunctivitis ("acute trachoma") coincidently with "trachoma (Prowazek) inclusions."

5. These bacilli in pure cultures present morphologically the same cycle of development as that shown by the "trachoma inclusions" seen in direct smears from cases of papillary conjunctivitis, and these direct smears also show transition forms between bacilli and inclusion. Therefore bacillus and inclusions are probably identical in these cases.

6. Among all of the species of micro-organisms isolated from cases of conjunctivitis only the gonococcus shows in pure cultures a cycle of development similar to that of the hemoglobinophilic bacilli; but the former presents certain characteristics corresponding to the characteristics found in the "trachoma inclusions" seen in direct smears from the host cases (gonorrheal ophthalmia neonatorum) which, coupled with the fact that transition forms between gonococci and inclusions are also seen in these smears, make it probable that inclusions and gonococci are identical in these cases.

7. "Trachoma inclusions," then, are simply intracellular nests of growing bacteria.

8. Among the other micro-organisms found in these cases certain staphylococci and certain of the non-hemolyzing streptococci may play an important part in the chronicity of certain cases, especially in cases of pannus.

Description of Plate

All Preparations Stained by Giemsa and Magnified about 1,000 *Diameters.*

Figures 1, 2, 4 and 5—Epithelial cells containing "trachoma inclusions" in films taken from conjunctiva in case of papillary conjunctivitis (formerly diagnosed acute trachoma).

Figures 3 and 6—Pure cultures of hemoglobinophilic bacilli from same case.

Figure 1—"Initial forms" of "inclusions" staining deep blue, and some showing as small bacilli.

Figure 2—Later stage of "inclusion" showing beginning appearance of reddish granules.

Figure 3—Twenty-four-hour culture on blood-agar of hemoglobinophilic bacilli showing coccoid forms similar in size and staining to those seen in early "trachoma inclusions."

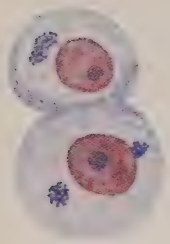
Figure 4—Later "trachoma inclusions" almost filling epithelial cell, showing many reddish granules and irregularly shaped light blue bodies.

Figure 5—Another later stage showing beginning absorption of the "inclusions."

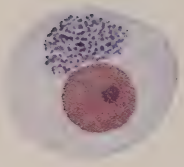
Figure 6—Four-day culture of the one shown in Figure 3, showing many reddish granules and irregularly shaped light blue masses similar in size and staining to those seen in later "trachoma inclusions."

Figures 7, 8, 10 and 11—Epithelial cells containing "trachoma inclusions" in film taken from conjunctiva in case of gonorrheal ophthalmia neonatorum.

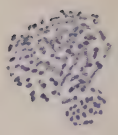
Figures 9 and 12—Pure cultures of gonococci from same case.



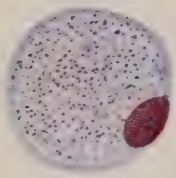
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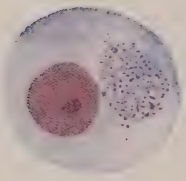
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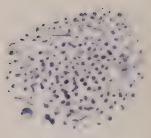
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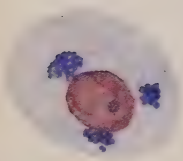
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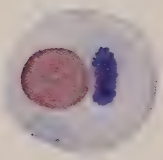
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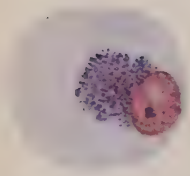
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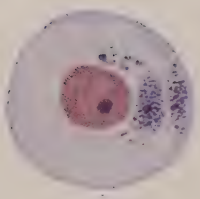
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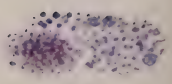
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Figure 7—Early stage showing morphologically typical cocci still at edge of "inclusion."

Figure 8—Later stage of "inclusion" showing dense group and beginning minute reddish granules.

Figure 9—Twenty-four-hour cultures of gonococci on blood-agar, showing staining and groups similar to those seen in epithelial cells in "early stage."

Figure 10—Later stage of inclusion" from gonorrheal case, showing minute reddish granules.

Figure 11—Still later stage of "inclusion" showing irregular bluish bodies and scattered minute reddish granules.

Figure 12—Three-day pure culture of gonococci showing minute reddish granules and swollen bluish bodies similar to those seen in "late inclusions."

III. DISCUSSION, SUMMARY AND CONCLUSIONS

By Anna Wessels Williams

The chief practical questions this study calls forth are: *First,* does a specific disease called trachoma exist in New York City? *Second,* if it does, how many individuals does it affect? and *third,* how contagious is it?

While our study has corroborated the opinion of others that there is no clear-cut clinical entity called trachoma, it has not ruled out the possibility of there being a specific disease answering in part, at least, to the classic descriptions of trachoma.

In considering the question of the present existence of such a disease in New York City, we must bear in mind several facts. First, since 1897 no case diagnosed as "active trachoma" has been allowed to enter New York City through its Port. Without doubt, this has lessened in a marked degree fresh foci of eye infections. Second, the poor in New York City (among whom most of the cases diagnosed as "trachoma" occur), live on the whole more hygienically than they do in their native countries where "trachoma" is said to be so frequent. Third, the methods of caring for the eyes of the citizens of New York City are becoming better year by year through the employment of various agencies such as school inspections and hygienic procedures, eye clinics, home visits, public lectures, and so on. The conditions resulting are directly preventive of the development of an infection from any case of "beginning trachoma," which either may have been infected from an "old case" or a "carrier," or may have slipped through the Port, as it might readily do even with the Port's strict oversight, if the majority of cases begin insidiously, as most authors think.

Under these conditions, therefore, we may still be having many new cases of "trachoma" which either abort or run a comparatively mild atypical course to cure.

Our studies so far show either that this conclusion is correct or that at present we have practically no cases of "trachoma" among the public school children on the lower East Side of New York City in a district supposed to be a hot bed of "trachoma."

For, among these 4,000 or more cases of conjunctival affections in the school children, under observation for an average of three years and seen from near the beginning of the disease, none have so far shown cicatricial formation except those among the few operated upon (expression) who show "operative scars." Moreover, the great majority of cases, after passing through a comparatively short chronic course, have presented, and still present, apparently normal conjunctivas.

That our cases have been under exceptional hygienic and medical treatment *from the beginning of the diseased conditions* may account in

great measure for this result. But whether they are cured light cases of "trachoma" or simply quiescent cases of "trachoma" ready to show specific exacerbation under certain conditions, or whether they are cases which were infected only with germs other than a specific "trachoma" germ, leaving their conjunctivas subject to reinfection and ready to progess, if later they become neglected, to "non-trachomatous pannus or cicatricial formation" or both, may only be answered by future study.

Among this large group of cases only eight during our period of observation have developed a condition diagnosed by ophthalmologists as pannus. Whether this condition in these case is a specific manifestation of a "trachoma" virus, we cannot say. All of these cases but one have now practically clear corneas and normal conjunctivas. All of them had, antedating and accompanying the pannus, more or less papillary conjunctivitis and follicle formation. From all of the eight curetted conjunctivas members of the pneumo-streptococcus group were obtained (p. 235). A polyvalent vaccine made from strains of the pneumo-streptococcus group seemed to have a quickening effect upon the march of these cases to recovery (p. 216). Whether or not this was simply coincidence is too soon to say.

Among our cases over three hundred showed a sluggish papillary thickening of the conjunctiva, accompanied in most cases by muco-purulent secretion and by marked follicle formation, apparently through the depths of the conjunctiva. The occurrence in these cases of "trachoma inclusions" and hemoglobinophilic bacilli at the height of the affection and irregularly throughout the course, coupled with the morphologic similarity of bacilli and "inclusion," has led us to the hypothesis that hemoglobinophilic bacilli growing in nests in the epithelial cells of the conjunctiva are identical with "trachoma inclusions" in these cases and are the cause of these subacute and chronic cases (papillary conjunctivitis with or without follicles), as well as of acute conjunctivitis. Whether or not this hypothesis is correct, the results from its practical application—*i. e.*, treating more minutely and following up all of our acute cases of conjunctivitis until we are sure of cure, as well as hunting up and treating acute cases in the families—gives evidence that it works.

The fact that we found "trachoma inclusions" in eight cases of acute catarrhal conjunctivitis, cured in an average of eight weeks, while transition stages between bacilli and inclusions in these and other acute cases were frequently seen, lends more evidence to this hypothesis.

There is no question, from our studies, but that bacteria other than hemoglobinophilic may grow in nests in the epithelial cells of the conjunctiva and so produce appearances like "trachoma inclusions," *e. g.*, gonococci in cases of gonorrhoeal ophthalmia (p. 232); and it is possible that we have missed a "specific trachoma bacterium" in some of our cases. But the the evidence seems strongly in favor of our hypothesis being true at least for the great majority of the cases of conjunctivitis in our public school children.

Furthermore, the evidence admits the possibility that neglected cases, or especially susceptible cases, infected with hemoglobinophilic bacilli may result in a fibrous tissue stage.

As regards the question of the degree of infectivity of these cases, again it is difficult to give a clear-cut answer owing to the variableness of the clinical picture.

On the whole, we have found that our schools at present seem to play an immaterial part in the spread of the chronic eye affections. For, among the 100,000 school children and over in the sixty schools we have studied more closely on the lower East Side, only 3,000, or thereabouts, have shown during the past three years more or less chronic affections of the conjunctiva, and over 2,000 of these have been only mild folliculosis. As for the more serious cases, no school stands out particularly as a focus of infection. The few schools that have the most cases have children coming from the most unhygienic families. In isolated instances in the past, infections apparently contracted in the classroom have been reported, but none have come under our observation during the past few years. The possibility of the spread of eye infections in schools may be reduced to a minimum by the employment of proper hygienic precautions such as have been carried out in Public School 21 (p. 188).

In regard to home infection, we have made estimates from two groups of cases. The first estimate, given by Miss Kurtz (p. 198), is made from the average chronic cases of papillary conjunctivitis. The percentage of probable infection was found to be 22.

In our other estimate made later, in which were included a number of the "old trachoma" cases (p. 181), as well as cases of papillary conjunctivitis. Miss Gurley found that in 87 families with an average of 7.5 individuals to the family (653 individuals), 144 individuals had a condition diagnosed by most ophthalomologists as trachoma. Fifty-seven of these, then, excluding the one in each family presumably infected from the outside, developed during the period of infection a chronic papillary conjunctivitis with follicles, that is about 10 per cent. of the 566 individuals exposed, a smaller proportion than that usually given.

In the families of the 38 pannus cases we have had (most of them among the chronic adult cases), a remarkably low percentage of cases of chronic conjunctivitis developed in the exposed individuals.

That our campaign in behalf of personal hygiene, both in home and school, which we carried on during the whole course of these studies, helped lessen the spread of infection from these cases, especially in the families, we think probable, not only from observations of isolated cases, but because at present so few subacute and chronic cases seem to be developing in our lower East Side district, while they are still reported as prevalent in other crowded sections of the city.

Unless we are able to exercise strict supervision over the cases of acute conjunctivitis occurring in schools and homes, and to carry out the school and home instructions in personal hygiene, we are convinced that after a time we may have large numbers of well-developed chronic cases, a certain percentage of which may finally show pannus and cicatricial changes. In short, they may present the picture seen at present in the neglected cases among the Kentucky Mountains and on some of our Indian Reservations.

Summary

1. The written descriptions of "trachoma" do not agree as to a clinical or pathologic entity.

2. Among the children of 60 public schools in the crowded lower East Side district of New York City (supposed to be a hotbed of "trachoma"), during the past four years we have found no cases of conjunctical affections answering in their entirety to the classic descriptions of "trachoma."

3. None of the 3,000 and more cases of follicular affections in these children have developed cicatricial changes due to infection; on the contrary, the great majority of them now present normal conjunctivas. We attribute these results chiefly to the following measures :

(*a*) The carrying out of preventive methods of treatment, such as ophthalmia schools and school clinics, summer camps, mothers' demonstration classes, home instructions and *follow-up work.*

(*b*) More minute care of acute, as well as of chronic cases, including the specific treatment given *chiefly at clinics* and the *follow-up work in the homes.*

(*c*) Non-operative procedures in a large number of cases formerly operated upon.

4. Hemoglobinophilic bacilli are found in a large proportion of the cases of chronic conjunctivitis (especially of the papillary type), as well as in those of acute catarrhal conjunctivitis (including clinically typical pink-eye).

5. These bacilli, though they probably represent several different varieties, have not yet been differentiated from *B. influenzae.*

6. These bacilli are found coincidently with "trachoma inclusions" in a high percentage of the cases of papillary conjunctivitis when examined from the beginning.

7. These bacilli show morphologic and staining characteristics similar to those seen in "trachoma inclusions;" and transition forms between bacilli and inclusions are frequently seen in these cases.

8. In a few cases of acute catarrhal conjunctivitis "trachoma inclusions" and "transition forms" have been often found coincidently with hemoglobinophilic bacilli, and in several other acute cases "transition forms" have been often found.

9. Many of the cases of chronic papillary conjunctivitis not actually followed from the beginning by us gave a history of repeated acute attacks, and several cases beginning with us as acute contagious conjunctivitis passed in the chronic condition called papillary conjunctivitis.

10. Cell inclusions similar to the "trachoma inclusions" found in the papillary conjunctivitis cases are found in cases of gonorrheal ophthalmia neonatorum, cultures from which show only gonococci. The inclusions differ somewhat from those found in the papillary conjunctivitis cases, and transition forms between gonococci and inclusions are seen in smears from the former cases.

11. Members of the pneumo-streptococcus group are frequently found in chronic as well as in acute cases, especially in those cases showing pannus.

Conclusions

1. "Trachoma inclusions" are nests of growing bacteria in epithelial cells—hemoglobinophilic bacilli in certain cases of papillary conjunctivitis, gonococci in certain cases of gonorrhoeal conjunctivitis and possibly other bacteria in certain other cases.

2. Under minute hygienic and medicinal treatment, such as is outlined in some of the preceeding articles, the great majority, if not all, of the cases of conjunctival affections of children may run a benign course resulting in normal conjunctivas.

3. Comparatively few, if any, cases of chronic conjunctivitis develop in individuals exposed if the rules of general and personal hygiene are carried out.

4. *If "trachoma" is present or should be introduced among our school children, it may be controlled by methods which are within practical limits.*

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A METHOD FOR DIFFERENTIATING COCAINE FROM ITS SUBSTITUTES

Halsey Durand

The widespread use of cocaine hydrochloride as a local anesthetic in the last ten years has stimulated manufacturing chemists in their efforts to put on the market a number of substitutes, which, while serving the same purpose, are said to be not so dangerous and not to produce the harmful after effects of cocaine. These substitutes may also be used to replace cocaine, the general use of which as a habit-forming drug has been prohibited by law, in an effort to deceive the chemist and nullify his testimony in criminal prosecutions for the possession and sale of the drug. At present there are at least eleven of these substitutes offered for sale. Some of these are so closely allied to cocaine and respond so nearly to the chemical reactions given by different authorities for cocaine that its identification is difficult if not quite impossible.

The writer has discovered that very characteristic reactions may be obtained and cocaine positively identified by the use of a di-sodium hydrogen phosphate solution.

The method of procedure is as follows:

The reagent is a saturated aqueous solution of di-sodium hydrogen phosphate, prepared by dissolving about 10 gms. of the salt in 100 ccm. of distilled water.

About 5 mgs. of the substance to be examined is placed in a small test tube (8 by 1 cm. is a convenient size) and dissolved in about 2 ccm. of water. It is advisable to run a control with each series of tests. About 0.5 ccm. of the reagent is added to each and the liquid sharply agitated. If the solutions are not sufficiently concentrated the precipitates or oily drops do not form at once and the test tubes should be allowed to stand for a few moments. The tubes are then shaken and a drop removed and placed on a microscope slide and examined with a 15 m. m. objective and a No. 2 eyepiece.

Cocaine can be easily identified by its very characteristic crystals.

The following is a summary of the behavior of cocaine hydrochloride and its substitutes:

I.—Cocaine hydrochloride—White crystalline precipitate. The crystals are elongated and irregularly placed.

II.—Alypin—White amorphous precipitate and oily globules.

III.—Anesthesin—No precipitate.

IV.—Apinol—No precipitate.

V.—Acoine—White amorphous precipitate.

VI.—Beta eucaine hydrochloride—No precipitate.

VII.—Euphthalamin hydrochloride—No precipitate.

VIII.—Holocaine hydrochloride—White crystalline precipitate. Distinguished from cocaine by its regular groups of crystals.

IX.—Novocaine—No precipitate.

X.—Nirvanine—White precipitate with a large number of oily globules and a small number of crystals.

XI.—Orthoform—No precipitate.

XII.—Stovaine—White precipitate, oily globules, no crystals.

Two other cocaine derivatives, alpha and delta cocaine, have been noted. These were not tested as they have no anesthetic properties.

The writer desires to gratefully acknowledge the valuable data received from Mr. Reginald Miller, of this laboratory, derived from the application of this test on several hundred samples received from the Police Department for identification preceding criminal prosecutions for the possession or sale of cocaine hydrochloride.

EXPERIMENTAL DATA ON THE DETERMINATION OF SACCHARIN IN FOODS WITH A MODIFICATION OF SCHMIDT'S METHOD

Halsey Durand

A recent ruling of the Board of Health of the Department of Health of New York City prohibited the use of saccharin in foods and drinks (see Section 68, Sanitary Code), the enforcement of which required the examination of a large number of samples of various foodstuffs and soft drinks in which violations were expected.

A method for the detection of saccharin was sought which could be done positively and with as much speed as possible.

A review of the literature gave five methods, as follows:

I.—Taste Test. The aqueous solution of the sample is acidified and shaken out with ether. The ethereal layer separated and evaporated to dryness and a small portion of the residue applied to the tongue. An extremely sweet taste followed by a bitter taste indicates saccharin.

II.—Bornstein's Test (Zeit. Anal. Chem. 27 S. 165). Aqueous solution is shaken as above and the ether residue is heated with resorcin and a few drops concentrated sulphuric acid, till the mass begins to swell. Cool. Repeat heating and cooling several times. Cool, dilute with water and neutralize with sodium hydrate. Red green fluorescence indicates presence of saccharin.

III.—Kastle's Test (Bull. No. 26. Hyg. Lab. U. S. Pub. Health & Mar. Hosp. Serv.). The ether residue is treated with a few drops of a mixture of 5 ccm. of phenol and 3 ccm. of pure concentrated sulphuric acid. It is then heated for 5 minutes to 160°-170°C. and the mass dissolved in water and made alkaline with sodium hydrate. A dark purplish red or pink indicates the presence of saccharin.

IV.—Schmidt's Test. (Rep. Anal. Chem. 30). Residue from ether extraction is heated in a porcelain dish with about 1 gm. sodium hydrate for one half hour at 250°C. in air oven or linseed oil bath. This converts saccharin to sodium salicylate. The melt is dissolved in water acidified with hydrochloric acid and shaken with ether. Evaporate ether, take up residue with water and add dilute ferric chloride solution. A violet color indicates the presence of saccharin: The absence of salicylic acid must first be ascertained before applying this method. If salicylic acid is present the ether residue is treated with dilute hydrochloric acid and bromine water added in excess. The liquid is well shaken and filtered. The salicylic acid is completely removed as a bromine derivative. The filtrate is made strongly alkaline and evaporated and heated as above. By this means the false saccharin frequently found in wines and giving faint reactions for saccharin is also eliminated.

V. Allen's Test. (Allen's Com. Organ. Anal., Vol. II., Pt. III., p. 38). The ether residue is rendered alkaline with sodium hydrate and ashed. The ash is taken up with water made acid with hydrochloric acid and tested with barium chloride for sulphur. The presence of sulphur indicates saccharin.

This test, a little potassium nitrate being added during ignition, is also used for the quantitative determination of saccharin. The barium sulphate being weighed and the weight multiplied by 0.783 = saccharin. This quantitative method was not tried as the presence of saccharin is sufficient ground for prosecution.

Test I.—The taste test was tried and found to be very satisfactory. The absence of the sweet taste being a good indication in most cases of the absence of saccharin. However, the possibility of other substances producing the sweet taste, and also of the sweet taste being masked by bitter substances in the residue, made it necessary to have some further test as confirmation.

Test II. was tried and gave good reactions. It was found, however, that a large number of organic compounds give the same reaction as saccharin, so this method was discarded.

Test III. was tried and excellent results were obtained when pure saccharin was used. When ether residues obtained from shaking out aqueous solutions of foods and drinks, were treated with the phenol-sulphuric acid mixture and neutralized with sodium hydrate the characteristic color did not appear. The failure of this reaction is evidently due to the presence of small amounts of impurity in the residue. Efforts were made to obtain the pure phenol compound from the aqueous solution after treating the residue with phenol-sulphuric acid mixture, by shaking out with amyl alcohol and isobutyl alcohol and treating the separated alcohol solutions with sodium hydrate, without results.

Test IV. (Schmidt's) was tried repeatedly by the laboratory force using the various modifications as suggested by different authorities. Invariably the ether residue, obtained by shaking out the acidified aqueous solution of the mass after heating, gave a benzoic acid reaction on addition of dilute ferric chloride solution. It was found also that the saccharin in the original ether residue was converted into benzoic acid when treated with saturated sodium hydrate solution and allowed to stand over night at room temperature. Excellent results were obtained in this way and this method was adopted by this laboratory as a confirmatory test to be used with the taste test. This method while giving good results required too long a time for its completion.

The writer has recently succeeded in obtaining the salicylic acid by Schmidt's Method (IV.), both in pure saccharin and in ether residues containing saccharin, by proceeding as follows:

100 ccm. of an aqueous solution of the sample to be examined for saccharin are acidified with phosphoric acid and shaken out with ether in the usual manner. The separated ether is filtered through double folded filter papers, to remove moisture, into a nickel dish of convenient size and evaporated to dryness. After ascertaining the absence of salicylic, see description of Schmidt's method, the residue is treated with about 1 ccm. of saturated sodium hydrate solution, allowing the alkali to come in contact with the entire residue. Place the dish on a piece of asbestos board about 3 mm. in thickness, heat for 8-10 minutes over a Bunsen flame, cool, take up with water, acidify with hydrochloric acid and shake out with

ether. Evaporate ether, to which a small amount of ammonium hydrate has been added, in a glass dish on water bath, take up residue with a little water and test with dilute ferric chloride for salicylic acid.

This method gave very satisfactory results requiring about three-quarters of an hour.

The writer has further simplified and shortened the time required for the method and also eliminated the possibility of the sublimation of the salicylic acid on the water-bath. This modification consists of omitting the treatment of the final ether residue with ammonium hydrate and its evaporation to dryness. The ether residue after separation is poured into a test tube and about 5 ccm. of water containing a small amount of ferric chloride is added. The test tube is then shaken vigorously and the ether and water layers allowed to separate. The presence of salicylic acid is indicated by a violent coloration of the water layer.

THE DETECTION OF GELATIN IN SOUR CREAM

ARMIN SEIDENBERG

The usual method for the detection of gelatin in food products such as milk or cream and that adopted provisionally by the United States Department of Agriculture* consists in the removal of the proteids with acid mercuric nitrate and the precipitation of the gelatin in the filtrate with picric acid. It, however, has been frequently noticed that a sweet cream which gave no precipitate with picric acid would, on souring, show quite a perceptible turbidity; often heavy enough to lead to the assumption that gelatin was present. It seems most probable that this is due to protein decomposition products not precipitated by the acid mercuric nitrate. G. E. Patrick, in an address on "The Detection of Thickeners in Ice Cream,"† held before the twenty-fourth annual convention of Official Agricultural Chemists, speaks of what is probably the same or a very similar decomposition product, often found in ice cream after it has soured. He is not certain as to the exact nature of this substance, but believes it to be due to the action of bacteria. He finds that this "psuedo-gelatin," as he calls it, forms a precipitate with picric acid similar to that formed by gelatin under the same conditions and mentions that there is no method known for distinguishing the one from the other. His suggestion that formaldehyde be added in the laboratory to prevent the bacterial action which produces this decomposition product is, of course, not applicable to creams received sour.

The picric acid precipitate from the sour cream is floculent, resembling $Al(OH)_3$ in form and settles quite rapidly; while that due to gelatin is small and granular, much like $BaSO_4$ in form, settles very slowly and will on shaking the test tube thoroughly coalesce in large lumps, leaving the solution almost clear. While these differences are quite well defined, the large number of sour cream samples received in this laboratory made a method leaving less room for doubt, in the detection of even small amounts of gelatin, very desirable. After some experimenting I found that there was a sharply defined difference between the solubilities of the two precipitates, in hot neutral water. While both precipitates are soluble on heating in slightly acid solutions, only the gelatin picrate is soluble in hot neutral water alone. The picric acid precipitate from the sour cream is seemingly entirely insoluble in hot water, after all the picric or other acid has been removed.

To 10 c. c. of the cream are added an equal volume of acid mercuric nitrate, the mixture shaken, 20 c. c. of water added, again shaken and filtered after five minutes. To the filtrate an equal volume of picric acid is added when, if gelatin or the soluble decomposition products from the sour cream are present, a precipitate is formed. This is the method mentioned in Bulletin No. 107 of the U. S. Department of Agriculture.

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* U. S. Dept. of Agric. Bulletin, 107 (rev.), p. 121.
† U. S. Dept. of Agric. Bulletin, 116, p. 24.

In order to definitely determine the nature of this precipitate, proceed as follows: Cork test tube, which should be a large one, and shake very thoroughly. In this way the gelatin picrate will coalesce and filtration be much more rapid. If possible, allow to stand for some time after shaking; most of the liquid can then be syphoned off. The precipitate being brought on to the filter is washed with water containing two to three drops of ammonium hydroxide to 100 c. c. until the washings are slightly alkaline to litmus, and then with neutral water until the washings are neutral to litmus. This will remove all excess of picric acid. The precipitate is then transferred to a small beaker and 10 to 20 c. c. distilled water added and heated to boiling. If the precipitate is very small in amount it need not be taken from the filter paper, but both can be boiled together.

Filter hot into a test tube. The filtrate will contain the gelatin picrate but not the precipitate derived from the proteids in the sour cream. Cool this filtrate and add an equal volume of picric acid when, if gelatin was present in the original cream, a decided precipitate will be formed. When large amounts of gelatin are present the solution may become turbid on merely cooling.

In order to determine the delicacy of this method 25 c. c. of a 1 per cent. solution of gelatin containing 0.25 grms. of gelatin were added to 50 grms. of sour cream. The sour cream thus contained only 0.5 per cent. of gelatin, which had little thickening effect on it. By using 20 to 25 c. c. of this cream instead of 10 c. c. as above, the presence of the gelatin could be determined with certainty. The above method has been in use. in this laboratory for some time and has been found to work satisfactorily, provided the conditions described are obtained. Even smaller quantities of gelatin than this can, of course, be detected by using a larger amount of the original sample.

ATTEMPTS TO IMMUNIZE AGAINST TUBERCULOSIS BY THE COMBINED ACTION OF INJECTIONS OF TUBERCULIN AND OF THE SERUM OF TUBERCULOUS RABBITS

James P. Atkinson and Charles B. Fitzpatrick

The blood of tuberculous animals was selected because it might possibly contain poisons or antibodies developed during the course of the disease which might immunize rabbits against tuberculosis. We injected also alternate doses of tuberculin with the view of obtaining if possible a more complete immunity and possibly a practical therapeutic serum against tuberculosis. All the available substances acquired by or embodied in the blood of the tuberculous animal during the onset and course of the disease until shortly before the death were thus utilized.*

The following experiments are given because of their intrinsic interest and because they show a specific difference in the action of the blood of normal and tuberculous animals.

If one injects a dog, *not previously sensitized* by an injection of tuberculin, intravenously with 3 c. c. of serum from a rabbit infected with tuberculosis, it does not suffer a reduction in arterial pressure when the serum is injected into the femoral vein and pressure taken in the carotid.

Dogs, however, sensitized by a subcutaneous injection of 5 c. c. of tuberculin, which is followed in from twelve to eighteen hours by an intravenous injection of 1.5 to 3 c. c. of tuberculous rabbit serum, do suffer a marked reduction in arterial pressure.

The depression result could not be obtained when the following sera were substituted for tuberculous sera: glanders (horse), typhoid (human), antityphoid (horse), antistreptococcus (horse), normal rabbit, normal horse, and a 2 per cent. solution of Witte's peptone. We have one record showing that the five-weeks-old tuberculous rabbit serum which did not cause the reaction in a sensitized dog, was reactivated by the addition of an equal amount of fresh rabbit serum, so that it caused a depression following its injection.

The subcutaneous injection of 10 c. c. of the tuberculous serum into three rabbits, each of which had been previously sensitized by an injection of 1 c. c. of crude tuberculin, caused death in from twenty-four to fifty hours.

Immunizing Experiments

The following experiments were carried out in our attempts to immunize against tuberculosis.

Experiment I.—A rabbit was given 1 c. c. of crude tuberculin as a sensitizing dose. Beginning two days later with two days intervening between each injection six injections of tuberculous rabbit serum were given, amounting in all to 10 c. c.

A rest of ten days was given the experimental rabbit, which was then given a fatal dose of a culture of bovine tuberculosis intravenously. Two

* The blood from which the serum was separated was obtained by bleeding tuberculous rabbits to death.

control rabbits were given an equal amount of the same culture. The controls lived thirty days, during which time they lost weight and presented a typical tuberculous appearance.

The autopsy showed marked and characteristic lesions of tuberculosis.

The experimental rabbit lived thirty-nine days and kept in good condition up to one week before its death and then lost weight rapidly. The autopsy showed the same marked and characteristic tuberculous lesions as the controls.

The fact of the long-continued good physical condition of the experimental rabbit encouraged us to repeat this experiment with some changes.

Experiment II.—Two rabbits were used in this experiment. One died after a fifth injection. Beginning January 8, 1910, four doses of 0.25 c. c. of crude tuberculin were alternated with doses of tuberculous rabbit serum, given two and three days apart; then six injections of tuberculous rabbit serum were given with two days intervening between the injections. One c. c of crude tuberculin and 34 c. c. of tuberculous rabbit serum were given in all.

A rest of 11 days was given the surviving rabbit, and on February 18, 1910, it was given a fatal dose of bovine tuberculosis intravenously. Two control rabbits were given the same amount of the bovine tuberculosis culture.

One control died April 5; the second control died July 11; both these rabbits showed marked and characteristic tuberculous lesions. The experimental rabbit died July 17, from pneumonia.

The autopsy showed that the lungs, liver, kidneys, suprarenal capsules, spleen and glands were free from tuberculous infection.

Both controls lost weight consistly until death.

The experimental rabbit held its weight up to a week before its death, when it lost weight very rapidly.

This experimental rabbit appears to have been successfully protected by injections of tuberculin and tuberculous rabbit serum from a fatal dose of a culture of bovine tuberculosis.

Experiment III.—One rabbit was given 6 injections of 0.25 c. c. of crude tuberculin alternated with 6 doses of 2 c. c. of tuberculous rabbit serum totaling 1.5 c. c. crude tuberculin and 12 c. c. tuberculous rabbit serum.

Experiment IV.—One rabbit was given 6 injections of 0.10 gm. of the alcohol insoluble residue of B. tuberculosis (prepared according to the method of Dr. V. C. Vaughan), which were alternated with 6 injections of 2 c. c. of tuberculous rabbit serum, totaling 0.6 gms. of the alcohol insoluble residue and 12 c. c. tuberculous rabbit serum. Common controls were made for Experiments III. and IV.

These four rabbits of Experiments III. and IV. with three controls were each given an intravenous injection of a lethal dose of bovine tuberculosis on July 22, 1910, after an interval of fourteen days since the last injection of tuberculous rabbit serum.

These rabbits all died and showed marked and characteristic lesions

upon autopsy except the rabbit which died on August 1, as the result of a bite, and one that died August 22 and was not autopsied. This animal which died August 22, however, showed the physical signs of tuberculosis before death.

The following table shows the time of death of the experimental rabbits and the controls.

Control 1—Died August 8.
Control 2—Died August 12.
Control 3—Died August 15.

Experimental Rabbits—Tuberculin and Tuberculous Rabbit Serum.

No. 1—Died August 12.
No. 2—Died August 22.

Experimental Rabbits—Tuberculosis Rabbit Serum and Insoluble Residue.

No. 1—Died from infected bite August 1.
No. 2—Died August 10.

These methods apparently did not produce any protection.

In order to test the properties of the products obtained from the treatment of the tubercle bacilli according to the directions of Dr. Vaughan the following tests were made.

Some of the soluble portion injected subcutaneously into a guinea pig produced the following symptoms: nervousness, weakness in the hindquarters, convulsions and death in about 45 minutes.

Injections of the alcohol—insoluble portion did not cause symptoms of any sort.

A small quantity of this insoluble portion was injected into each of three guinea pigs. After three weaks two of these pigs were injected intraperitoneally with an emulsion of the dead tubercle bacilli obtained from the preparation of the tuberculin from which the split products had been prepared, with the following results:

Pig No. 1. After thirty minutes, restlessness followed by weakness in the hind legs; violent convulsions followed by extreme fatigue. These convulsions continued during the day and the pig died finally in a convulsion six hours after the injection.

The autopsy showed some undissolved bodies of tubercle bacilli spread over the omentum.

Pig No. 2. Some restlessness, but no other sign that it was affected by the injection. Two days later this pig was seized with a convulsion and died.

The autopsy showed some undissolved bodies of tubercle bacilli on the omentum, not spread over it but collected together in small groups. The edge of the spleen was covered with small abscesses which did not show tubercle bacilli.

The serum of two rabbits treated according to Experiment No. 2 acquired a clumping property for the tubercle bacilli. This was shown by each of the rabbits.

An emulsion of tubercle bacilli (bovine type) was mixed with clear serum obtained from these animals six days after the last injection.

A mixture of this serum with a uniform suspension of the tubercle bacilli, after standing overnight at room temperature, was found to cause well defined clumping. The control in normal sodium chloride solution was not clumped.

The following two attempts to immunize were also made.

Experiment V.—Two rabbits were given 4 injections of 0.25 c. c. of crude tuberculin alternated with 4 injections of 3 c. c. of tuberculous rabbit serum, followed by 5 injections of tuberculous rabbit serum, totaling 1.25 c. c. of crude tuberculin and 27 c. c. of tuberculous rabbit serum.

Blood taken from the ear vein of these experimental rabbits on September 28 clumped a uniform suspension of the tubercle bacillus on standing overnight.

The control in normal sodium chloride solution was not clumped. These rabbits were inoculated with 0.01 mg. of a virulent culture of the bacillus of bovine tuberculosis with three controls on October 4, 1910.

The animals died as follows:

Experimental white rabbit died December 3, 1910; lived two months.

Experimental brown rabbit died November 8, 1910; lived one month, four days.

Control Rabbit No. 1 killed (bled to death); lived one month, six days.

Control Rabbit No. 2 died November 19; lived one month, fifteen days.

Control Rabbit No. 3 died December 14; lived two months, ten days.

Experiment VI.—A series of small injections were also given as follows: Five injections of 0.01 c. c. crude tuberculin were alternated with injections of 1 c. c. of tuberculous rabbit serum followed by injections of tuberculous rabbit serum; totaling 0.05 c. c. tuberculin and 31.5 c. c. tuberculous rabbit serum.

The experimental rabbit died of general tuberculosis after one month and sixteen days, following a gradual loss of two-thirds of its original weight. This rabbit then was not protected by the injection of small doses of tuberculin and tuberculous rabbit serum.

Conclusions

1. There is present in the blood of rabbits and probably other animals suffering from tuberculosis a substance or substances which cause a depression in the arterial pressure of dogs sensitized with tuberculin. This substance or substances have a fatal effect upon rabbits sensitized with tuberculin when 10 c. c. of the serum is injected subcutaneously.

2. Injections of tuberculous rabbit serum in repeated sublethal doses into sensitized rabbits produced in one case an apparent increase in the animal's resistance (Experiment I.).

In another case apparently complete protection was produced (Experiment II.). In several other cases no protection could be observed (Experiments III., V., VI.).

3. The serum of rabbits sensitized with tuberculin and injected with tuberculous rabbit serum acquire the power to clump a uniform suspension of the tubercle bacillus.

4. Injections of the alcohol insoluble portion of the B. tuberculosis (prepared as directed by Dr. V. C. Vaughan) into a sensitized rabbit did not produce protection in the two animals tested.

5. Guinea pigs may be sensitized with Vaughan's alcohol insoluble residue of the B. tuberculosis to subsequent injections of this product.

6. The power of tuberculous rabbit serum to lower the arterial pressure in sensitized dogs and to kill sensitized rabbits may be practically utilized as an additional diagnostic method for tuberculosis.

IS SERUM ANAPHYLAXIS A DANGER OF SUFFICIENT IMPORTANCE TO LIMIT OUR USE OF PROTECTIVE SERA IN THE TREATMENT OR PREVENTION OF DISEASE?

William H. Park

The antitoxin treatment of diphtheria soon brought to light that the injection of the whole serum or of the proteids of horse serum into man was sometimes followed by certain disagreeable effects.

These, after a first injection, usually became manifest after a number of days, while after a later injection they appeared within a few hours or days.

The name of serum sickness has been applied to the reaction since the classical work upon this subject in 1907 of von Pirquet and Shick.[1]

The usual reaction is not of serious importance except in young and very sick children, where the rise of temperature, urticaria, etc., may lower the resistance. In a few cases the symptoms are more pronounced, there being added albumin in the urine, joint complications, swelling of mucous membranes and intense scarlatiniform rashes. It is important to realize that these disagreeable after effects are nearly as marked after a first injection as following later ones. The heating used and the elimination of much of the serum proteids in the refining of the diphtheria and tetanus antitoxins has largely eliminated the more severe types of this common form of serum sickness following their use. The sera used in other diseases besides tetanus and diphtheria are not as a rule refined and their injection is still accompanied by the usual percentage of severe serum reactions.

The object of this paper is to consider not this usual form of serum sickness, but the occasional cases of collapse in which death may occur within a few minutes after the injection of serum. While these cases are classed with those of serum sickness their pathology is not fully understood. They occur as often after a primary injection as after later ones. Death probably takes place only when the anaphylatic shock occurs in an abnormal individual, such as one suffering from the condition of status lymphaticus or subject to asthma.

H. F. Gillette[2] who became interested in these cases through a personal experience made an investigation and was able to collect in 1909 23 cases of which 10 died. Of these 23 cases, 16 gave a history of respiratory troubles. Rosenau[3] and Anderson obtained the serum used in two of the fatal cases and found the sera to have unusually toxic properties.

The reason for these sudden deaths has received various explanations. The symptoms in most are like those of anaphylaxis. Pricking and burning sensations frequently occur first and then spasms and failure of respiration, while as a rule the heart continues for a time to beat at

[1]Die Serumkrankheit Wien, 1907.
[2]Jour. A. M. A., 1913, LII, 580.
[3]Hygienic Lab. Bulletin No. 45, p. 45.

a rapid rate. Erythematous rashes are sometimes marked. The frequent history of previous asthmatic attacks led Schultz and Jordan to suggest as a partial explanation of the severity of the symptoms an abnormal development of the smooth muscle of the bronchi, the contraction of which is believed to cause bronchial spasms, while the work of Rosenau and Amoss indicates as a possible cause of the anaphylactic reaction the sensitization through inhalation of proteid material exhaled by horses. The deaths, when they occur, are so sudden and unexpected that they make a great impression upon those who witness them and also upon those who read or hear of them. This has brought it about that many advise that immunization against diphtheria be stopped so as to eliminate danger if a serum injection is needed in future years. This, I believe, is a great mistake for immunization is often a great protection and as a matter of fact these fatal cases are extremely rare.

The experience of physicians in the employ of the Department of Health with the use of serum in the hospitals and among the poor is of the utmost value in estimating the frequency of these reactions. All cases of diphtheria treated in the Department Hospitals since January 1, 1895, have received diphtheria antitoxin either in the whole serum or in a solution of globulins.

During this time there have been over 30,000 patients treated without a single death due to anaphylaxis and with only two cases of severe collapse. Both these recovered completely. The average amount of antitoxin and serum for each case has been approximately 10,000 units and 10 c. c. of serum.

During the past five years all cases of scarlet fever admitted to the hospitals have received an immunizing dose of one c. c. of antitoxin globulin. The amount of original serum averaged 3 c. c. Over 16,000 have received antitoxin and very many a second injection at a period in which they were sensitized and not a single case of collapse has occurred.

In the city at large since 1895, the inspectors have given immunizing or curative doses to about 105,000 persons. Two deaths followed the injections and these were both from immunizing injections. In one of these an autopsy was obtained; a marked case of status lymphaticus was found. The records of the inspectors give no information as to cases of collapse which recovered. Private physicians have reported to me three cases of deaths and two of collapse. How many others have occurred in private practice and what the proportion is to all cases treated, we have no means of estimating. It is because of knowing the total cases treated as well as the total fatal accidents that the extensive records of the Department are so valuable.

It is very important to note that every death followed a primary injection. It is of course true that the primary injections greatly exceeded the repeated injections. There were two cases of temporary collapse after second injections given intravenously. A study of these figures clearly proves that the cases of collapse and death are so rare that they should not prevent the use of serum, where it is really indicated

and that the fear of sensitizing and so producing dangerous possibilities in the future is practically groundless when moderate sized subcutaneous injections are given and not great when large injections are given cautiously. The fact that a person has received a first injection without serious reaction would cause me to give him a second subcutaneous injection with less fear of a fatal result than in one never injected.

It is true that most of these cases will have within twelve hours a slight rise of temperature and if a rash develops it is apt to occur early rather than late, but unless one watches the cases closely it is unlikely that the observer would suspect whether the cases received a primary or a repeated injection.

In order to study the results following second injections, a number of cases under observation were given varying amounts with the following results.

Reaction Following a Second Serum Injection in Sensitized Persons

Number Persons	Amount 2d Injection		Number Having Temp. Rise	None	Reaction	
	Antitoxin	Globulin Solution			Urticaria	None
15	10,000 units	(10 c.c.)	6	9	10	5
9	5,000 "	(5 ")	5	4	2	7
9	1,000 "	(1 ")	3	6	1	8
4	500 "	(0.5 ")	3	1	0	4

These second injections were all given at a time when the cases were highly sensitized. Twenty-six of the persons were treated between the thirtieth and seventieth day after the first injection. The urticarias were all mild and none of the children appeared sick. They followed the injection in from two to eighteen hours. The rise of temperature was in most less than two degrees Fahr., but in three it was from 2.5 degrees to 3.5 degrees.

The number of slight reactions 17 in 37 is high, but many of those were so slight that they would have been overlooked except for the unusually close observation given them.

Others report similar experiences. Goodall states that in 63 cases given a second injection between 35 and 365 days, there were three severe and 14 moderate reactions. There were no cases of collapse.

Nemmser* studied histories of 1,000 receiving a second injection, and 102 three or more injections. None of these 1,102 cases suffered from collapse or death.

The intracutaneous test has been suggested to disclose dangerous liability to anaphylaxis. Moss, in 1910, drew attention to the value of the intracutaneous test to discover persons liable to shock. He found

* Deutsch. Med. Woch., 1913, XL, 740.

that five out of 21 persons who had previously received serum injections responded within a few hours with a local reaction. With the assistance of one of the resident physicians of the Hospital for Infectious Diseases, Dr. Archibald Wood, I tested a number of previously injected cases and found that they regularly developed an immediate local reaction. When given a serum injection nearly fifty per cent. showed either temperature or urticaria, or both, but none were severe. Dr. Wood gave an intracutaneous test to fifty consecutive patients on their admission to the hospital for diphtheria. Three of these showed an immediate reaction. Each received 10,000 units in about 8 c. c. of globulin solution. All three developed urticarial rashes and a rise of temperature within twelve hours, but in none were the symptoms severe.

The information gained was misleading rather than helpful as a guide to injections. The test might be valuable in cases in which a history of asthma was present or in a second injection in which the indications for treatment were not pressing. Here an absence of reaction would lead us to use the antitoxin.

Preventive Measures.

A number of investigators have shown that large doses of chloral, atropine or ether will prevent the development of shock. The doses to be effective must be very large and would probably be more dangerous than the slight peril they were used to obviate. The use of preparations containing a large number of units in each c. c. of serum in divided doses seems to be the best available practical measure. The first subcutaneous injection should be not over 0.2 c. c. as we do not know the limits of safety. The cases of sudden death have happened as frequently after 2 c. c as after larger doses.

If no striking general reaction occurs within one hour, a second injection of 0.2 c. c can be given and if this be well borne, in another hour 0.4 c. c. This same amount can then be repeated several times if necessary. It is easy now to procure globulin solutions with 3,000 units to each c. c.

We are now able to obtain human serum containing as much as 50 units of antitoxin per c. c. This could be used to immunize asthmatic cases.

INFLUENCE OF PROTEIN CONTENT ON THE ABSORPTION OF ANTITOXINS AND AGGLUTININS INJECTED SUBCUTANEOUSLY

William H. Park, Lemuel W. Famulener and Edwin J. Banzhaf

The subject of this communication has become important because of the fact that diphtheria and tetanus antitoxins are supplied in this country most commonly in the form of the refined and concentrated globulin preparation. The methods employed in the United States are those developed in this laboratory by Atkinson, Gibson and Banzhaf. These antitoxic preparations contain that fraction of the serum protein designated pseudoglobulin. In the actively immunized horse, the antitoxins are intimately associated with the pseudoglobulin, and, by the process of "salting out," may be separated with it from the other proteins, such as albumin, euglobulin, etc. By these methods, a concentrated preparation of diphtheria antitoxin, for instance, may be prepared containing 2,000-3,000 units per cubic centimeter with a protein (pseudoglobulin) concentration of 18-20 per cent., starting with an original serum containing 400-600 units and a protein content of 8-9 per cent. This concentration of antitoxin is obtained about equally by the elimination of the non-antitoxic proteins and by the abstraction of water. The removal of the water is merely a convenience, while the removal of the proteins together with the changes produced by the heating employed in the process removes certain deleterious substances which are present in the whole serum. Careful observations of cases in the hospital wards under our supervision have shown that serum reactions are less frequent in patients receiving the refined antitoxin than in those who have had the whole serum product.

In the treatment of diphtheria, it is a well recognized fact that the antitoxin should reach the general circulation as quickly as possible after its administration. The injection of the antitoxin directly into the blood stream is the only way that fully meets such requirements, but owing to the technical difficulties encountered, this method is not commonly employed by the practitioner. Instead, the subcutaneous injection is the means of administration in general use. In connection with the subcutaneous method of administration, this question has arisen: Does the highly concentrated antitoxic preparation, with its increased protein content, pass as rapidly from the subcutaneous or muscular tissues to the blood as do preparations having a larger volume of fluid of lower protein and lower antitoxin concentration? This question becomes of special interest in connection with certain commercial preparations of concentrated antitoxin containing a percentage of protein sometimes reaching three and one-half times that of the native antitoxic serum.

The influence on absorption of the protein concentration in the antitoxin solutions was considered by us when we commenced to use the refined antitoxin. The results of our investigations convinced us that a concentration to at least double that of an immunized horse serum had little, if any, appreciable effect in retarding absorption. The results ob-

tained by L. E. Walbum* with the absorption of agglutinin in rabbits which showed that in those animals in which the protein concentration of the injected fluid was equal to that of the blood serum the agglutinin was more rapidly absorbed than from solutions containing either a higher or lower protein concentration, caused us to attempt a new investigation of the subject.

Walbum states at the commencement of his paper, that on account of work carried out by Henderson-Smith and E. Tobin, he assumed that the conditions affecting the absorption of antitoxins were similar to those affecting the absorption of agglutinins. He went even further and assumed that the conditions affecting the absorption in the subcutaneous tissues of rabbits of *B. Coli* agglutinin produced by the goat and contained in goat serum or goat globulins, were similar in the case of the absorption of horse produced antitoxin from the tissues of man. In somewhat condensed form, his conclusions are as follows: The absorption of agglutinin from the subcutaneous tissues is much slower from serum containing 15 per cent. of protein than from that containing the normal amount (6.2 per cent., goat). When the more concentrated protein solution is diluted with normal saline solution, so as to be reduced to the same protein content as the normal, the agglutinin is absorbed equally from both solutions. Less than the normal concentration retards the absorption from subcutaneous tissues, but increases the rate of absorption from that injected into the muscles. The absorption from the peritoneal cavity is not affected by the protein concentration of the serum.

A critical examination of Walbum's results gives the impression that the difference in rapidity of absorption of the agglutinin from the solutions of different protein concentration was less marked than Walbum judged. Thus, if the rabbit receiving the concentrated protein in his experiment 1 is compared with the rabbit receiving the normal protein percentage in the experiment 7, the absorption curves are practically identical, if allowance is made for the different weights of the animals. The similarity of the rate and degree of absorption of the agglutinin from solutions of different protein concentrations is strikingly shown in Walbum's experiment 10. We reproduce his table (1) simply adding figures (in brackets) correcting the amounts to correspond with a weight of 1950 grams.

The figures in this table show that in this experiment the increased protein concentration had no appreciable effect in retarding the rate of absorption or the amount. While the lessened protein concentration appeared to decrease the rate and amount of absorption after the first day, yet it apparently increased both in the earlier and more important period.

Original Investigations.—As it is possible that the sera of different animals vary in their absorption characteristics, we determined to test the effect of protein concentration upon absorption by using the serum of the horse because this is the animal almost universally employed to furnish protective sera for man. We also, because of the importance of this question and the lack of danger of the experiment, tested the matter in man as well as in animals.

* Ztschr. f. Immunitätsf, etc., 1912, XII, 546.

TABLE I

VERSUCH X.—SUBKUTANE INJEKTION (WALBUM)

	14.95 Proz. Eiweiss Kaninchen, 1950 g.		10 Proz. Eiweiss Kaninchen, 1950 g.		7 Proz. Eiweiss Kaninchen, 1700 g.		4 Proz. Eiweiss Kaninchen, 1700 g.		1 Proz. Eiweiss Kaninchen, 1750 g.	
		Aggl.-Einh. in 1 c.cm. Ser.		Aggl.-Einh. in 1 c.cm Ser.		Aggl.-Einh. in 1 c.cm. Ser.		Aggl.-Einh. in 1 c.cm. Ser.		Aggl.-Einh. in 1 c.cm. Ser.
Vor der Injektion.....	>0.2		>0.2		>0.2		>0.2		>0.2	
16 Std. nach der Injektion.	0.03	33.3	0.0225	44.4	0.0185	54 [47]	0.015	67 [58.4]	0.013	77 [69.1]
26 " " " "	0.007	143	0.007	143	0.007	143 [124.6]	0.005	200 [174.3]	0.007	143 [128.3]
40 " " " "	0.0035	286	0.003	333	0.0029	340 [296.4]	0.004	250 [217.9]	0.0055	182 [163.3]
50 " " " "	0.0029	345	0.00275	364	0.0025	400 [348.7]	0.0037	270 [235.3]	0.004	250 [224.3]
64 " " " "	0.0025	400	0.00225	444	0.0021	476 [414.9]	0.0035	286 [249.3]	0.0037	270 [242.3]
74 " " " "	0.0026	384	0.0022	455	0.002	500 [435.8]	0.0035	286 [249.3]	0.0037	270 [242.3]
88 " " " "	0.00275	364	0.00225	444	0.0021	476 [414.9]	0.0037	270 [235.3]	0.004	250 [224.3]
98 " " " "	0.004	250	0.0025	400	0.0024	417 [363.5]	0.005	200 [174.3]	0.0049	204 [183]
136 " " " "	..	...	0.004	250	0.0035	286 [249.3]	..	...	..	...

Our first experiments to test the question of absorption of antitoxin in different protein concentrations were performed upon goats. Nine animals, which were divided into two main groups, were selected for the tests. Serum samples taken for controls before beginning the experiments showed no appreciable amounts of antitoxin. Each animal received 10,000 units of diphtheria antitoxin by subcutaneous injection in the lower abdominal region. The antitoxin was given incorporated with widely different concentrations of protein (pseudoglobulin), and sufficient NaCl was added to make isotonic (0.8 per cent.). Different volumes of the mixtures also were used in the injections. Two animals received a volume of 2.7 c. cm. containing 30 per cent. solids; three animals, 10 c. cm. volume, 30 per cent. solids; while one received 30 c. cm. volume, 30 per cent. solids. Three animals received solutions having only 7.5 per cent. total solids. As mentioned above each mixture contained 10,000 units of diphtheria antitoxin, and in six of the cases the animals were injected in one place only. While in the remaining three instead of injecting in one place, the dose was equally distributed in three places in each animal. Blood samples taken from each animal at intervals after the injection had the serum removed by clotting out, and the antitoxic value of each was determined for the following periods: 18, 36, 48, 72 and 96 hours. As a basis for comparison the antitoxic value in units per c. cm. was computed relative to an animal weighing 50 pounds and the results tabulated as shown in Table II.

TABLE II

Influence of Protein Concentration Upon Absorption of Antitoxin

Goats (Subcutaneous Injection)

	10,000 Units Diphtheria Antitoxin								
	Solids, 30 per cent. (Protein, 29.2 per cent+NaCl, 0.8 per cent)						Solids, 7.5 per cent. (Protein, 6.7 per cent+NaCl, 0.8 per cent.)		
Number.....	1 H.	2 H.	3 H.	4 H.	5 H.	6 H.	1 L.	2 L.	3 L.
Vol. injected..	30 c.c.	10 c.c.	10 c.c.	10 c.c.	2.7 c.c.	2.7 c.c.	10 c.c.	10 c.c.	10 c.c.
Places injected	3	3	1	1	1	1	3	1	1
After injection	units	units	units	units	units	units	units	units	units
18 hrs.	7.82	7.86	6.8	5.7	8.37	5.58	8.22	8.75	7.68
36 hrs.	10.2	9.07	8.83	7.6	8.64	6.97	10.72	9.0	8.4
48 hrs.	10.2	8.47	9.12	7.6	8.91	7.16	10.01	8.5	7.92
72 hrs.	8.84	8.47	9.12	7.6	8.64	6.51	9.65	8.0	7.2
96 hrs.	6.8	6.05	7.41	5.7	7.56	6.51	7.86	6.0	7.2

The antitoxic unit strength was first estimated between points of 0.25 units difference. In correcting the figures to a uniform goat weight (50 lbs.) the figures show apparently more accurate estimation.

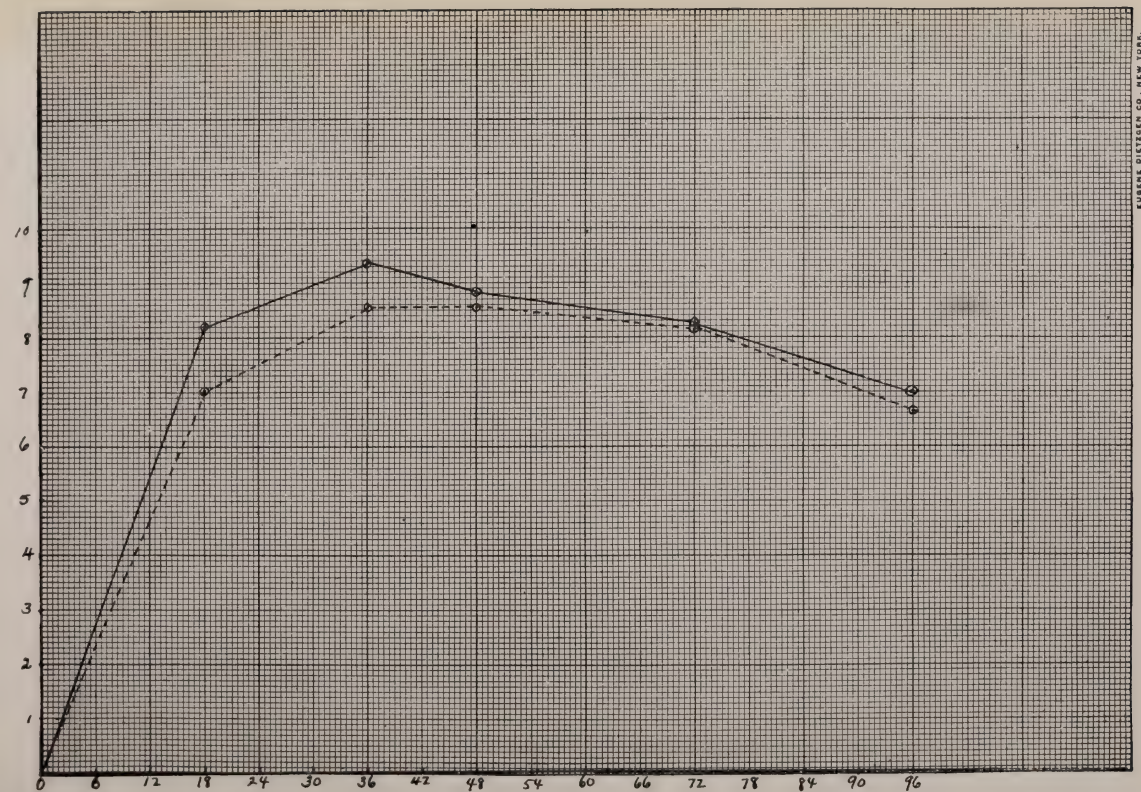


CHART I.—Comparative absorption from high and low protein concentrations.
..... = average antitoxin content of goats receiving high protein concentration.
——— = average antitoxin content of goats receiving low protein concentration.

The antitoxic unit strength was first estimated between points of 0.25 units difference. In correcting the figures to a uniform goat weight (50 lbs.), the points of estimation necessarily show different intervals. The weights of the animals were as follows, given in order from left to right: 68, 60.5, 57, 47.5, 54, 46.5, 71.5, 50 and 48 lbs., respectively.

Owing to individual irregularities in absorption, the average values were taken for all the animals which received antitoxin with low solid content, and for those which received antitoxin with high solids, and comparison were made between the two series (Chart I). It was found that the degree of absorption was somewhat greater for the low solid content mixtures throughout, especially the first period (18 hrs.), in which it was approximately 15 per cent. higher; this difference gradually diminished so that at the 36th hr. period, it was less than 5.5 per cent., and at 48 hrs. 2.7 per cent.

A second series of tests were carried out upon normal men. Two men received subcutaneous injections of 4.5 c. cm. diphtheria antitoxin containing 10.000 units and 18 per cent. of total solids. Two men were given the same dose of antitoxin (10,000 units) to which an equal volume of physiological salt solution had been added, thus doubling the volume (9 c. cm.), and reducing its protein percentage one-half. The dose was injected subcutaneously in one place. Bleedings were taken at definite intervals after the injection. The antitoxin content was determined for each blood sample, then the unit value per cubic centimeter was computed to a standard weight (100 lbs.) for comparative purposes. Slight amounts of normal antitoxin were subtracted, when present, for purposes of tabulation. These results are shown in Table III.

TABLE III

Men. (Subcutaneous Injections)

Injected in One Place	10,000 Units Diphtheria Antitoxin			
	Solids, 18 per cent. (Protein, 17.2 per cent+NaCl, 0.8 per cent)		Solids, 9.4 per cent. (Protein, 8.6 per cent+NaCl, 0.8 per cent)	
Subject.....	R——.	S——.	J——.	F——.
Volume injected	4.5 c.c.	4.5 c.c.	9 c.c.	9 c.c.
After injection .	units	units	units	units
3 hrs.	0.45	0.52—	0.12+	0.27+
6 hrs.	0.72	0.91	0.25	0.55
9 hrs.	1.17	1.04	0.31	0.82
24 hrs.	2.70	3.38	0.93	1.86
48 hrs.	3.15	4.03	2.0	2.76
96 hrs.	3.15	4.29	2.0+	2.76—

The unit value of each serum sample in this table has been computed to a standard weight of 100 lbs. for purpose of direct comparison. Weights of men reading from left to right are, 180 lbs., 260 lbs., 125 lbs., and 138 lbs.

In these experiments it was found that when the weight was considered the degree of absorption was greater in the men who received the concentrated preparation. This is the opposite of the findings in the tests on goats. Considerable variation of weight existed between the different men, the extremes were 125 lbs. and 260 lbs. Believing that the weight factor and other individual characteristics influenced the above result, it was decided to reinject two of the men. Two of the subjects, one large and one small, were selected from the group and used for reinjection. About nine weeks after their first injection each one received a second subcutaneous injection (in one place) of 10,000 units of diphtheria antitoxin from the same lot as used in the first series of tests. But, in this case, the smaller man, instead of the larger, received the 4.5 c. cm. volume of 18 per cent. total solids preparation. Blood samples were taken from each and the antitoxic content of the serum was determined.

Upon comparison, it was found that the absorption of antitoxin in each man corresponded very closely to the results shown in the first test (Table IV). Therefore, it appears that in this series of tests, the rate and degree of absorption were largely independent of the percentage of protein present within the limits used, but were individual characteristics depending upon the subject receiving the injection. The persistence of the antitoxin in the blood is not affected by the protein concentration.

TABLE IV

Men (Subcutaneous Injections)

Injected in One Place	10,000 Units of Diphtheria Antitoxin			
Subject	F———.		S——.	
Volume Injected....	2d Injection 4.5 c.c.	1st Injection, 9.0 c.c.	1st Injection, 4.5 c.c.	2d Injection, 9.0 c.c.
Concentration	Solids, 18 per cent. (Protein, 17.2 per cent+NaCl 0.8 per cent)	Solids, 9.4 per cent. (Protein, 8.6 per cent+NaCl 0.8 per cent)	Solids, 18 Per cent. (Protein, 17.2 per cent+NaCl 0.8 per cent)	Solids, 9.4 Per cent. (Protein, 8.6 per cent+NaCl 0.8 per cent)
After injection	units	units	units	units
24 hrs.	1.86	1.86	3.38	3.12
48 hrs.	2.96	2.76	4.03	3.64
72 hrs.	2.82	....	....	3.9
96 hrs.	2.82	2.76	4.29	3.9

High and low protein concentrations in the same individual.

After completing the series of experiments upon man and goats, we decided to do similar experiments upon rabbits using a specific agglutinin, thus taking a type of antibody and species for test animal, which would allow us to compare our results with those of Walbum. A specific agglutinin for *B. typhosus* derived from an immunized horse, was fractionated and the pseudoglobulin separated with a portion of the agglutinin; this after dialysis was further concentrated in a desiccator over H_2SO_4 at 36-37 degrees C. The concentrated product contained 20 per cent. solids, including 0.8 per cent. NaCl, and had double the agglutinating value of the original agglutinating serum. The original agglutinating serum contained 9.88 per cent. total solids. Three groups of three animals each were given subcutaneous injections in one place (over the abdomen) as follows: (1) 16 c. cm. original agglutinating serum (low solids); (2) 8 c. cm. volume, conc. agglutinin preparation (high solids); (3) 16 c. cm. volume, composed of 8 c. cm. conc. agglutinin preparation 8 c. cm. normal pseudoglobulin containing the same percentage of total solids. Bleedings were made at intervals after injection, and the usual quantitative tests for agglutinins were made upon the clotted out serum.

In those animals which received the concentrated preparation, a local reaction occurred at the point of injection followed by infiltration, and, in several animals, a necrosis of the surrounding tissues. As is evident, this reaction greatly disturbed the normal absorptive function. However, it may be of interest to note that when the results of the tests were averaged for each of the three groups (Table V), and curves plotted, they roughly paralleled each other; each group simply showing different degrees of absorption. The first group of animals which received the original agglutinating serum gave only a slight local reaction and showed the highest degree of absorption of the specific agglutinin. The second group which received 8 c. cm. of the conc. preparation showed less absorption throughout, while the third group with the 16 c. cm. volume, high solids, was still lower. These results would indicate that an infiltration about the injected area must also be an important factor in retarding the absorption of antibodies. The latest period at which the tests were taken revealed that the different sera had approached each other in agglutinating strength. There was slight bacterial contamination of the injected sera. It is not thought that this influenced the local reaction, but it is possible that it did.

Conclusion

The degree of protein concentration which is usually employed to produce the refined and concentrated diphtheria antitoxic globulin preparations has little or no effect in retarding the absorption of the antitoxin from the subcutaneous tissues. The removal of water, if not pushed too far, is therefore a justifiable means of lessening the quantity of fluid to be injected.

Any preparation which caused local inflammatory reaction lessens the rate of antitoxin absorption.

TABLE V

Rabbits (Subcutaneous Injections)

Typhoid Agglutinins, 80,000 Units	Group I			Average	Group II			Average	Group III			Average
Weight of Animals	1560 g.	1570 g.	1500 g.	1543 g.	1550 g.	1570 g.	1510 g.	1543 g.	1530 g.	1600 g.	1485 g.	1538 g.
Agglutinin Preparation	Low Solids	Low Solids	Low Solids		High Solids	High Solids	High Solids		High Solids	High Solids	High Solids	
Volume	16 c.c.	16 c.c.	16 c.c.		8 c.c.	8 c.c.	8 c.c.		16 c.c.	16 c.c.	16 c.c.	
Local Reaction	Very Slight	Very Slight	Very Slight		Marked	Moderate	Marked		Marked	Intense	Intense (Abscess)	
After injection	Aggl. U.	Aggl. U.	Aggl. U.		Aggl. U.	Aggl. U.	Aggl. U.		Aggl. U.	Aggl. U.	Aggl. U.	
6 hrs	60	70	60	63	40	60	40	46	30	40	10	26
12 "	120	145	120	128	80	135	100	105	60	90	40	63
24 "	200	245	250	231	150	235	205	196	150	170	90	136
36 "	250	345	350	315	200	335	280	271	250	240	125	205
48 "	300	420	400	373	275	385	330	330	300	315	150	255
60 "	350	470	450	423	300	415	380	365	350	340	175	288
75 "	400	520	400	440	350	485	380	405	400	365	200	321
100 "	325	470	350	381	325	460	330	371	350	390	225	321

The average weight of the rabbits comprising each of the three groups being practically identical the measure of the agglutinating strength of the serum taken from the individual rabbits has not been corrected for a common weight. Instead the average strength of each group has been added.

THE FREQUENCY OF B. TUBERCULOSIS IN THE MARKET-MILK OF NEW YORK CITY

MARIE GRUND AND HARRIET L. WILCOX

During the summer of 1912 we examined 120 specimens of milk for the presence of tubercle bacilli. The samples were taken indiscriminately from the large 40-quart cans in which the loose market-milk reaches New York City. Collections were made at the railroad depots by the regular Milk Inspectors of the Health Department, 15-20 c. c. being forwarded to the Research Laboratory in the ordinary screw-cap bottles.

The milk, which was on an average 36 hours old, was centrifuged for 15 minutes, at a speed of 2,400 revolutions. Two guinea pigs of nearly uniform size were used in testing each sample. One of these received 1 c. c. of the cream, the other the same amount of the sediment, subcutaneously into the left groin. At the end of about seven weeks we gave each surviving pig a subcutaneous injection of 2 c. c. of tuberculin, after the suggestion of Anderson. None of the pigs which survived this dose showed any tuberculosis, but in addition to the undoubtedly tubercular animals, four pregnant females succumbed. Careful search disclosed no evidence of tuberculosis, and these four pigs were accordingly classed among the negative cases, the more so since the other pigs inoculated with the same four samples of milk were also free from any lesions.

An outbreak of guinea-pig septicæmia during the summer months complicated matters, and at the end of the experiment we found that in only 78 out of the total 120 cases examined, both pigs survived long enough for the establishment of tubercular changes. Since in 33⅓ per cent. of our positive cases only one pig contracted tuberculosis, it seems better to include in our calculations only those cases in which both cream and sediment pigs survived long enough to make a diagnosis. Among the 78 samples of milk 9, or 11.5 per cent., contained virulent tubercle bacilli.

This is a little lower than the figure found by Dr. Hess for the New York City milk (16 per cent.). Whenever unselected market milk has been examined, the percentages found by other observers vary between wide limits, from Ostertag's 2 per cent. to the 55.5 per cent. for Berlin market milk, examined by Proskauer, Seligman and Croner (in the latter instance only 9 samples were examined).

Of the eighteen pigs inoculated with the nine positive specimens, two died before the end of two weeks; three did not react to tuberculin and showed no lesions at autopsy; one showed suspicious lesions, which could not, however, be proven to be tubercular, as the pig reinoculated with portions of the suspected lymph node died acutely of septicæmia. Twelve of the eighteen pigs showed definite tubercular changes.

In addition to the macroscopic findings, we based our diagnosis on the demonstration of the tubercle bacillus in smears. Reinoculations were unsuccessful owing to the epidemic of guinea-pig septicæmia, and for the same reason cultural work could not be carried out. Pure cultures were obtained in two cases, but no attempt was made to determine the type of tubercle bacillus.

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A BACTERIOLOGICAL AND SANITARY STUDY OF CREAM AS SUPPLIED TO NEW YORK CITY

M. Catherine Schroeder

In October, 1911, a study of the New York City cream supply was taken up under the supervision of Dr. Wm. H. Park, Director of the Bureau of Laboratories. The investigation covered the following points:

(1) The bacteriological content of cream, both raw and pasteurized;

(2) The conditions governing its storage and sale;

(3) The conditions surrounding the production, manufacture and shipment of cream to New York City.

Samples were taken from 35 of the principal dealers in cream, who received their supply from 86 different creameries. The cream from which these samples were taken was shipped from the following states: New York, Massachusetts, Vermont, Ohio and Pennsylvania.

It was planned to procure samples from as many different sources as possible from the city dealers. These samples of cream were accordingly taken from cans and bottles, at wholesale and retail plants and from stores and wagons. They represented the output of the dealers to wholesale and family trade for manufacturing purposes. The total number of samples taken was 468. Twelve of these samples being discarded for various reasons, there were left 456 samples for bacteriological examination. In taking samples for analysis, it was found that ordinary aluminum pipettes could not be used owing to their narrow calibre, so special pipettes were devised. These pipettes were made of glass 21 inches long and ½ inch in diameter with one end tapered to a narrow opening ¼ inch in diameter. This type of pipette facilitated the withdrawing of the heavy creams from the cans and thus insured an even sample. The cream was placed in small sterile bottles, iced thoroughly and conveyed to the laboratory for examination.

The grades of cream commonly sold in New York City are known as light sweet-cream, medium sweet-cream, heavy sweet-cream, light sour-cream and heavy sour-cream. The number of samples procured of each of these grades is given in the following table (I.):

TABLE I

Type of Cream.	No. of Samples.	Type of Cream.	No. of Samples.
Light sweet-cream, raw....	58	Light sweet-cream, past....	25
Medium sweet-cream, raw..	5	Medium sweet-cream, past..	3
Heavy sweet-cream, raw....	177	Heavy sweet-cream, past...	168
Light sour-cream	11	Heavy sour-cream	9
Total number of samples.....			456

Method of Examination.—At the Laboratory the creams were recorded and the bacteriological analysis made. In making this analysis the samples were shaken 25 times and plated on 1½ per cent. nutrient agr. The dilutions used were 1-10,000 and 1-100,000. The plates were incubated at a temperature of 80 degrees Fahr. for three days. The results obtained are shown in Table II.

Judging from the results of the bacteriological examinations set forth in Table II. and also from a large series of presumptive tests for *B. coli,* it would seem that the bacterial development in a considerable proportion of cream is very great. Thus of the raw light-cream 66 per cent. of the samples contained over 1,000,000 bacteria per c. c.; 19 per cent. of the pasteurized cream samples showed the same bacterial content.

The bacterial content of the heavy sweet-creams, whether labeled raw or pasteurized, was about the same. This averaged for all samples 10,000,000 per c. c. The sour cream naturally had an enormous number of bacteria. It is necessary, in considering these figures, to remember that the fat globules as they rise, to form cream, either naturally or in the separator, carry the bacteria with them to the top. Therefore, cream contains per volume at least three times as many bacteria as the whole milk from which it was obtained.

The sanitary investigation concerning the conditions governing the sale of the cream, revealed a lack of definite information on the labels or tags attached to the containers from which the cream was sold. In a number of cases, containers were found without label of any kind. Again, if labeled, no information was given concerning the important points of pasteurization, such as the method employed, the temperature used, or the date of pasteurization. Thus one of the largest dealers in cream, shipping thousands of pounds to New York City, pasteurized cream at one station, shipped it to a second station, where it was mixed with other cream shipments, and again pasteurized for the third time and placed in barrels. It was then held over until a sufficient amount had accumulated and then shipped twice a week, by express, to two large milk concerns. None of these interesting data appeared on the tags. In 205 cases, the dealers stated that they were without information as to pasteurization, 23 containers bore tags showing shipping points only, while 40 gave absolutely no information whatever.

In tabulating the data concerning the age of the cream reckoned from the date of shipment, it was found that out of the 468 samples no data were obtainable in 195 (41.64 per cent.) cases. The age of the cream was stated in only 273 (58.33 per cent.) cases. Of those in which the age was stated, it varied from one to five days from the date on the label. This is shown in Table III.

TABLE II

Table of Bacteriological Content of Cream

Creams as Labeled	Less than 10,000 Bacteria per C. C.		10,000 to 100,000 Bacteria per C. C.		100,000 to 1,000,000 Bacteria per C. C.		1,000,000 to 10,000,000 Bacteria per C. C.		10,000,000 to 50,000,000 Bacteria per C. C.		50,000,000 to 100 Mill. Bacteria per C. C.		100 Mill. or Over Bacteria per C. C.		Total No. of Samples	Average Bacterial Count per C. C.
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.		
Raw, light sweet-cream	1	1.72	4	6.8	14	24.1	31	53.4	6	10.3	2	3.4	....	.....	58	10,655,504
Past., light sweet-cream	1	3.8	3	11.4	17	65.3	4	15.3	1	15.3	....	.....	....	.....	26	1,371,503
Raw, heavy sweet-cream	1	.59	16	9.5	30	17.7	77	45.8	34	20.0	10	5.9	....	.....	177	10,251,258
Past., heavy sweet-cream	15	8.9	28	16.0	47	28.1	31	18.5	40	23.9	5	2.9	2	1.1	168	9,874,166
Raw, med. sweet-cream	....	.....	2	40.0	1	20.0	2	40.0	....	.....	....	.....	....	.....	5	4,764,000
Past., med. sweet-cream	....	.....	....	.....	3	100.0	....	.....	....	.....	....	.....	....	.....	3	460,000
Light sour-cream	....	.....	....	.....	....	.....	2	18.1	9	81.8	....	.....	....	.....	11	126,954,545
Heavy sour-cream	....	.....	....	.....	1	11.1	4	44.4	4	44.4	....	.....	....	.....	9	87,485,142

TABLE III

Total No. of Samples	Age of Cream													
	No. Not Stating Age		No. Stating Age		Less 24 Hours Old		24 to 48 Hours Old		2 to 3 Days Old		3, 4 and 5 Days Old		Over 5 Days Old	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
468	195	41.6	273	58.3	147	53.4	109	39.9	9	3.2	5	1.8	3	1.0

The average temperature of creams sold in cans was 39.1 degrees Fahrenheit; the average temperature of the creams sold in bottles was 40 degrees Fahrenheit.

The sanitary conditions under which the cream was kept and sold in the city were good (with one exception), most of it being stored in refrigerators or ice-houses. These places were cleaned at least twice a week and were in good condition when visited. As a rule, the cream was well iced; the one exception was where the cream was being soured for the trade, the cans were in a dirty vat, in a room which was also dirty and contained rubbish and filth.

Simultaneously with the above mentioned study, an investigation of the conditions existing at the creameries from which the cream was shipped was also made. As previously stated, the cream examined had been shipped from 86 different creameries. Of these 76 were inspected and data were obtained in 71 cases. These data are shown in Table IV.

TABLE IV

SANITARY CONDITIONS SURROUNDING THE PRODUCTION, STORAGE AND SALE OF CREAM

	No.	Percent.
Creameries investigated.....Total	76	100.00
Creameries receiving milk daily	59	77.61
Creameries receiving milk every second day	15	19.73
No data obtained as to receipt of milk	2	2.63
Creameries manufacturing milk products:		
Cream	74	97.36
Butter	35	46.05
Cheese	26	34.20
Milk Sugar	12	15.79
Casein	25	32.89
Skim Milk	4	5.24
Milk Powder	2	2.63
Buttermilk	12	15.79
Concentrated Milk	2	2.63
Fortified Milk	1	1.31
Condensed Milk	12	15.79
Confectioners' Comp	6	7.89
Ice Cream	3	3.95
Candy	1	1.31

TABLE IV.—*Continued*

	No.	Percent.
Method of Separation of Cream:		
Centrifugal	45	59.20
Gravity	5	6.57
Tubular Method	1	1.31
No data obtained	27	35.52
Pasteurization of Cream:		
No. of creameries where cream is pasteurized	46	60.52
No. of creameries where cream is not pasteurized	28	36.84
No data obtained	2	2.63
Pasteurized by holder process	22	28.95
Pasteurized by flash process	10	13.16
Pasteurized by steam jet placed directly into cream	7	9.21
No data obtained	9	11.84
Cream repasteurized	11	14.47
Cooling of Cream:		
Properly cooled	53	69.73
Not properly cooled	11	14.47
No data obtained	12	15.79
Labeling of Cream Shipments:		
Properly labeled	41	53.94
Not properly labeled	12	15.79
No data obtained	23	30.26
Shipment of Cream:		
Shipped daily	29	38.15
Held over	26	34.20
Not shipping	1	1.31
No data obtained	20	26.31
Shipping to dealers	64	84.20
Shipping to consumers	—	——
Shipping to other creameries	11	14.47
Cream handled in sanitary manner	29	38.15
Cream not handled in sanitary manner	37	48.38
No data obtained	9	11.84
Scores obtained by creameries for methods:		
No. receiving less than 25 points	7	9.21
No. receiving from 25 to 30 points	13	17.10
No. receiving from 30 to 35 points	14	18.42
No. receiving from 35 to 40 points	7	9.21
No. receiving from 40 to 45 points	11	14.47
No. receiving from 45 to 50 points	19	24.99
No data obtained on	5	6.57
Scores obtained by creameries for construction:		
No. receiving less than 25 points	0	——
No. receiving from 25 to 30 points	1	1.31
No. receiving from 30 to 35 points	2	2.63
No. receiving from 35 to 40 points	6	7.16
No. receiving from 40 to 45 points	14	18.42
No. receiving from 45 to 50 points	48	63.14
No data obtained on	5	6.57

To briefly summarize Table IV., the following points may be noted: In forty-five (59.2 per cent.) cases, the cream was separated by the centrifugal method; pasteurization of cream was carried on in forty-six (60.5 per cent.) creameries; at eleven (14.49 per cent.) re-

pasteurization of cream took place. In forty-eight and six-tenths per cent. of the creameries, the cream was not handled in a sanitary manner during the process of manufacture and shipment. In sixty-nine and seven-tenths (69.7 per cent.) of the cases, the cream was properly cooled and in fifty-three and nine-tenths per cent. (53.9 per cent.) the cream was properly labeled. In regard to the shipment of cream, daily shipments were made in twenty-eight and two-tenths per cent. (28.2 per cent.), thirty-four and two-tenths per cent. (34.2 per cent.) of the cream was held over one or more days, one large dealer making only two shipments per week. In their score on methods, forty-four and seven-tenths per cent. (44.7 per cent.) of the creameries received less than thirty-five out of a possible score of fifty.

Conclusions

(1) Judging from the results of the study of the bacteriological examination, there remains much to be done in the way of protecting cream from bacterial contamination.

(2) That the sanitary conditions surrounding the sale of cream in the city are fairly good.

(3) That the methods used in handling cream during the process of manufacture leave much to be desired from a sanitary point of view.

I am indebted to the following persons for their assistance in the different phases of the work: Messrs. E. J. McCullen and T. Schroeder and Misses McDermott and Schweitzer.

A REPORT UPON THE BACTERIOLOGICAL CONDITION OF THE MILK SUPPLY OF NEW YORK CITY DURING THE YEAR 1912

M. Catherine Schroeder

During the year 1912, the Department of Health of New York City inaugurated the system of grading milk according to its quality, and the conditions surrounding its production and its sale. The sanitary code was accordingly revised to that effect. The grades of unmodified milk were determined as follows:

Grade A. For Infants and Children

1. *Certified milk, raw* (N. Y. County Medical Society).
   Handled with special care, and taken from cows negative to tuberculin test. The milk shall not contain more than 10,000 basteria per c. c.
   Guaranteed milk, raw (N. Y. Department of Health).
   Handled with special care and taken from cows negative to tuberculin test. The milk shall not contain more than 30,000 bacteria per c. c.
2. *Inspected milk* (*raw*) from cows negative to the tuberculin test. The milk shall not contain more than 60,000 bacteria per c. c.
3. *Selected milk pasteurized* from cows not subjected to the tuberculin test; physical test only required. The milk shall not contain more than 200,000 basteria per c. c. before pasteurization and not more than 50,000 bacteria per c. c. prior to delivery to the consumer.

Grade B. For Adults

1. *Selected Milk, Raw*
   Only physical examination of cows required. The milk shall not contain an excessive amount of bacteria when delivered to the consumer.
2. *Pasteurized Milk*
   No tuberculin or physical test of cows required. The milk must not contain an excessive number of bacteria before pasteurization.

Grade C. For Cooking and Manufacturing Purposes only

1. *Heated milk* not satisfying the requirements of Grade B.
2. Condensed Skim Milk.
3. Condensed or concentrated whole milk.

This grading of the milk was considered so important that some of the inspectors were withdrawn for a time from the bacteriological force

and assigned to aiding the farmers in getting their dairies into the conditions necessary to meet the new requirements. The general bacteriological work in the country was considerably reduced therefore, and the taking of samples mainly confined to the type of milk comprising the great bulk of the supply, namely, "Grade B Raw," and "Grade B, Pasteurized."

Pasteurization of City Milk

The fact that a number of large epidemics had been traced to the use of raw milk and the conviction that the problem of supplying a "safe raw milk" for the consumer in New York City had grown overwhelmingly great, made it seem wise for the Board of Health to strongly urge the pasteurization of milk. This measure has resulted in the reconstruction of many plants for this purpose. The bacteriological examinations of samples of pasteurized milk is steadily becoming more important, and special tests have been adopted.

Bacteriological Analysis of Milk Taken at Country Creameries

There were 27,935 samples taken. Of this number, the results obtained from the examination of 8,297 samples are shown in Table 1. A study of this table shows that of the 8,297 samples, 4,564, or 55 per cent. showed a bacterial content of less than 10,000 per c. c.; 2,052, or 24.73 per cent. contained from 10-50,000 bacteria per c. c.; 635, or 7.6 per cent. had from 50-100,000 per c. c.; 799, or 9.5 per cent. contained from 100,000 to 1,000,000, and 247, or 2.98 per cent. contained over a million. Contrasting these results with those obtained in 1911, gives us the following interesting facts also shown in Table I. Thus, in the "less than 10,000 bacteria per c. c." group, we have 55 per cent. in 1912, against 36.8 in 1911; in the "10-50,000" group, we have but 24.73 per cent., against 31.34 per cent. in 1911.

Dividing the samples into two groups: Those with a bacterial content below 50,000 per c. c., and those with a bacterial content above 50,000 per c. c., gives us the following result:

Year	Bacterial Count	Number of Samples	Per Cent.
1911	Less 50,000 per c.c.	14,389	70.76
1912	" " " "	6,606	79.73
1911	Over 50,000 per c.c.	5,945	29.23
1912	" " " "	1,681	20.27

The curves obtained by a comparison of percentages of the number of samples with less than 50,000 bacteria per c. c. is shown in figure one.

The temperatures of the milk as delivered at the creamery are shown in Table 2, where the comparisons with the temperatures of the milk for 1911 are also shown.

TABLE 1

Months		Total Number of Samples	Bacteria per C.C.									
			Less than 10,000		10—50,000		50—100,000		100,000—1 Million		1,000,000 and Over	
			Number of Samples	Per Cent.	Number of Samples	Per Cent.	Number of Samples	Per Cent.	Number of Samples	Per Cent.	Number of Samples	Per Cent.
January	1911	1,274	579	45.44	472	37.	128	10.	91	7.14	4	0.3
	1912	1,251	864	69.06	259	20.70	88	7.03	39	3.11	1	.07
April	1911	1,140	673	59.	289	25.14	78	6.78	94	8.17	6	.52
	1912	268	134	50.	77	28.7	29	10.8	28	10.4	...	...
May	1911	2,097	874	41.95	580	27.84	303	14.54	293	14.06	47	2.25
	1912	40	5	12.5	8	20.	6	15.	20	50.	1	2.5
June	1911	2,099	621	29.58	689	32.82	206	9.8	410	19.53	173	8.24
	1912	329	155	47.1	105	31.9	30	9.1	36	10.9	3	.91
July	1911	830	299	35.88	239	28.6	132	15.84	130	15.6	30	3.6
	1912	264	158	59.8	45	17.0	22	8.3	31	11.7	8	2.6
August	1911	1,393	332	23.81	340	24.38	181	13.18	319	22.67	221	15.85
	1912	850	209	24.4	263	30.7	123	14.4	176	20.6	79	9.2
September	1911	2,152	550	25.5	533	24.76	298	13.84	525	24.39	246	11.43
	1912	1,387	634	45.7	282	20.3	103	7.4	249	17.9	119	8.6
October	1911	2,802	1,135	39.72	934	32.59	315	11.02	358	12.53	60	2.1
	1912	1,115	539	48.4	331	29.6	96	8.6	117	10.4	32	2.8
November	1911	2,514	988	39.29	1,032	41.05	308	12.25	180	7.15	6	.23
	1912	701	456	65.04	152	21.6	48	6.8	41	5.8	4	.5
December	1911	1,326	445	33.55	491	37.02	195	14.71	184	13.87	11	.82
	1912	2,092	1,410	67.2	530	25.3	90	4.3	62	2.9	...	...
Year	1911	17,627	6,496	36.8	5,599	31.8	2,144	12.16	2,584	14.63	804	4.55
	1912	8,297	4,564	55.	2,052	24.73	635	7.6	799	9.5	247	2.98

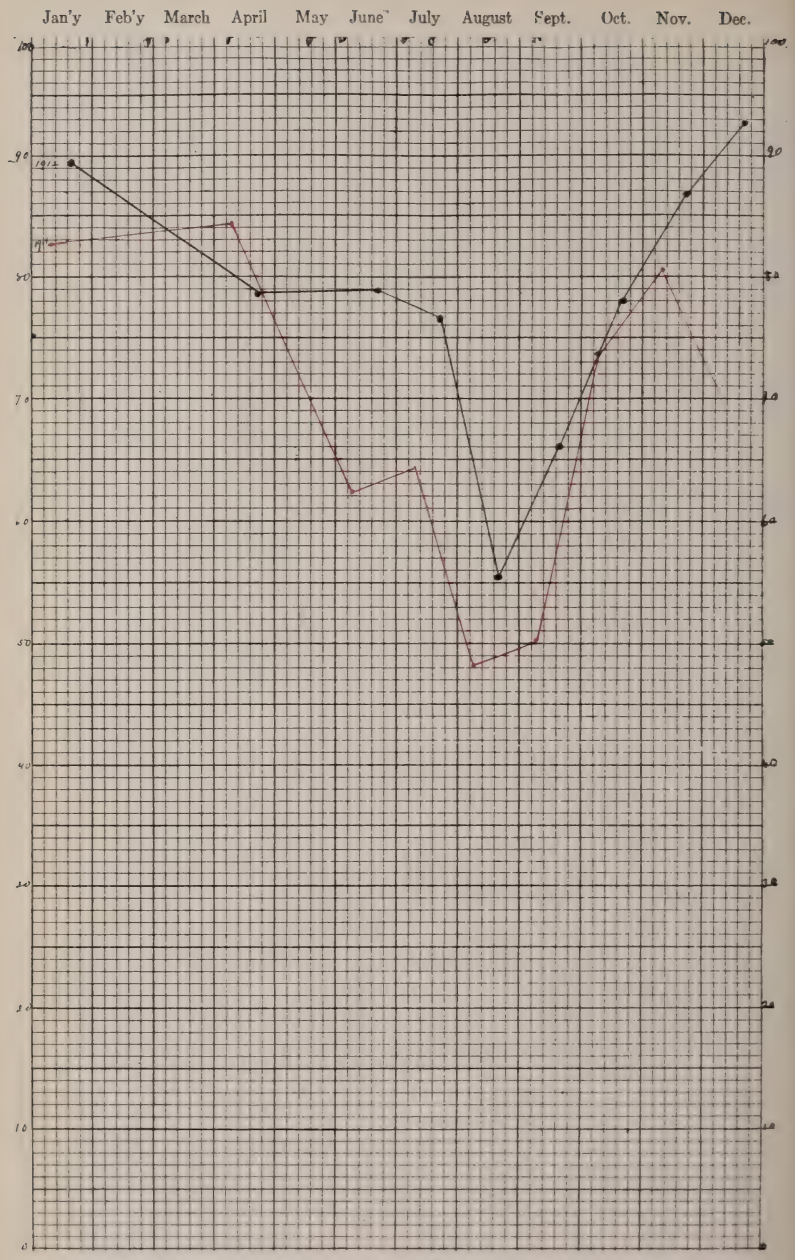


FIG. I.—Curves showing percentages of samples with less than 50,000 bacteria per c. c for years 1911-1912.

Grouping the temperatures into two divisions, those below 50 degrees and those above 50 degrees Fahr. gives the following tabulation:

Year	Degrees Fahrenheit			
	Under 50°		Over 50°	
	Number	Per Cent.	Number	Per Cent.
1911	7,335	39.21	11,363	60.79
1912	3,316	44.27	4,170	55.73

This again shows a slight improvement for 1912.

Having thus concluded the review of the results obtained in sampling the milk at country creameries, let us review briefly the bacterial content of the samples taken in the City of New York from milk in the course of transportation or held for sale.

In making an analysis of the samples, the plan originated last year was followed, namely, of taking the samples obtained during one representative month of each quarter of the year. The number and kind of samples taken, together with the month in which they were taken are shown in the table below:

Month	Type of Container	Number of Samples
January	Raw milk in cans	612
	" " " bottles	570
	Pasteurized milk in cans	60
	" " " bottles	266
April	Raw milk in cans	993
	" " " bottles	748
	Pasteurized milk in cans	146
	" " " bottles	437
July	Raw milk in cans	409
	" " " bottles	543
	Pasteurized milk in cans	72
	" " " bottles	448
October	Raw milk in cans	467
	" " " bottles	907
	Pasteurized milk in cans	102
	" " " bottles	689
	Total	7,469

The relative gains and losses are shown graphically in Chart II, where the grades and years are contrasted. It is gratifying to note that on the whole, the year's results show an increase of the number of low counts and a decreasing number of high counts.

The Pasteurization of Milk

As previously stated, during the year 1912, pasteurization of milk was introduced as a general measure for insuring a "safe" supply of milk for New York City. While information gathered concerning the bacteria which survived pasteurization would be interesting, it was considered that the presumptive test for B. Coli would be adequate to show that suf-

ficient destruction had occurred to insure no typhoid bacilli passing and this together with the number would indicate

1. Sufficiency of pasteurization.
2. Effectiveness of the holding system.
3. Presence or absence of contamination in bottling.
4. Effectiveness of the washing of cans and bottles.

The medium used for this purpose was the lactose neutral red solution in fermentation tubes.*

Method of Testing the Efficiency of Pasteurization

For the purpose of pasteurization, the milk is placed in the mixing vat and the machinery is started. As the milk passes on its way, four samples of milk are taken from each portion of the machine to be tested; also from bottles or cans of milk ready for shipment and from cans or bottles which are supposed to have been cleansed and sterilized, and are ready to be filled with milk. These samples are brought to the laboratory and the milk diluted to the following proportions:

Raw Milk—1-100 and 1-1,000.

Past. Milk—1-1,000, 1-100, 1-10 and 1 c. c.

Regular agar plates are made for the quantitative test.

These dilutions are then inoculated into lactose neutral red tubes.* The inoculated tubes are then incubated for 3 days at a temperature of 37 degrees C. and the gas and color changes noted.

In a small proportion of tests, when the presumptive test for B. Coli was positive, further tests for the identification of the organisms were carried out to confirm the accuracy of the presumptive test.

The following method of procedure was used:

Determinative Tests for B. Coli

Endo or Conradi plates were streaked from the lactose neutral red medium and incubated for 24 hours.

Plates examined, colonies counted.

Typical colonies fished, hanging drops made and examined.

Smears made, stained and examined.

Tubes of Sugar Media inoculated and incubated for 3 days at 37 degrees C.

Tubes examined and recorded.

Indol and Vosges tests made.

The bacterial reduction due to pasteurization is shown in Table 2.

This method of testing the process of pasteurization of milk has been *in use* for over two years and has given general satisfaction.

Judged from the data thus shown, it would seem that the bacterial content of milk during the year 1912 shows a considerable improvement over the condition of 1911.

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* *Method of Preparing the Lactose Neutral Red Solution*

Stock Solution { 10 gms. peptone
5 gms. sodium chloride
1,000 c. c. distilled water.

Boil, filter through paper into sterile flasks.
Autoclave for one-half hour.
To stock solution add 5 c. c. of a 5 per cent. watery solution of neutral red and 10 gms. of lactose.
Place in Arnold sterilizer for 10 minutes to dissolve, filter, tube.
Sterilize in Arnold sterilizer for two successive days for 20 minutes.

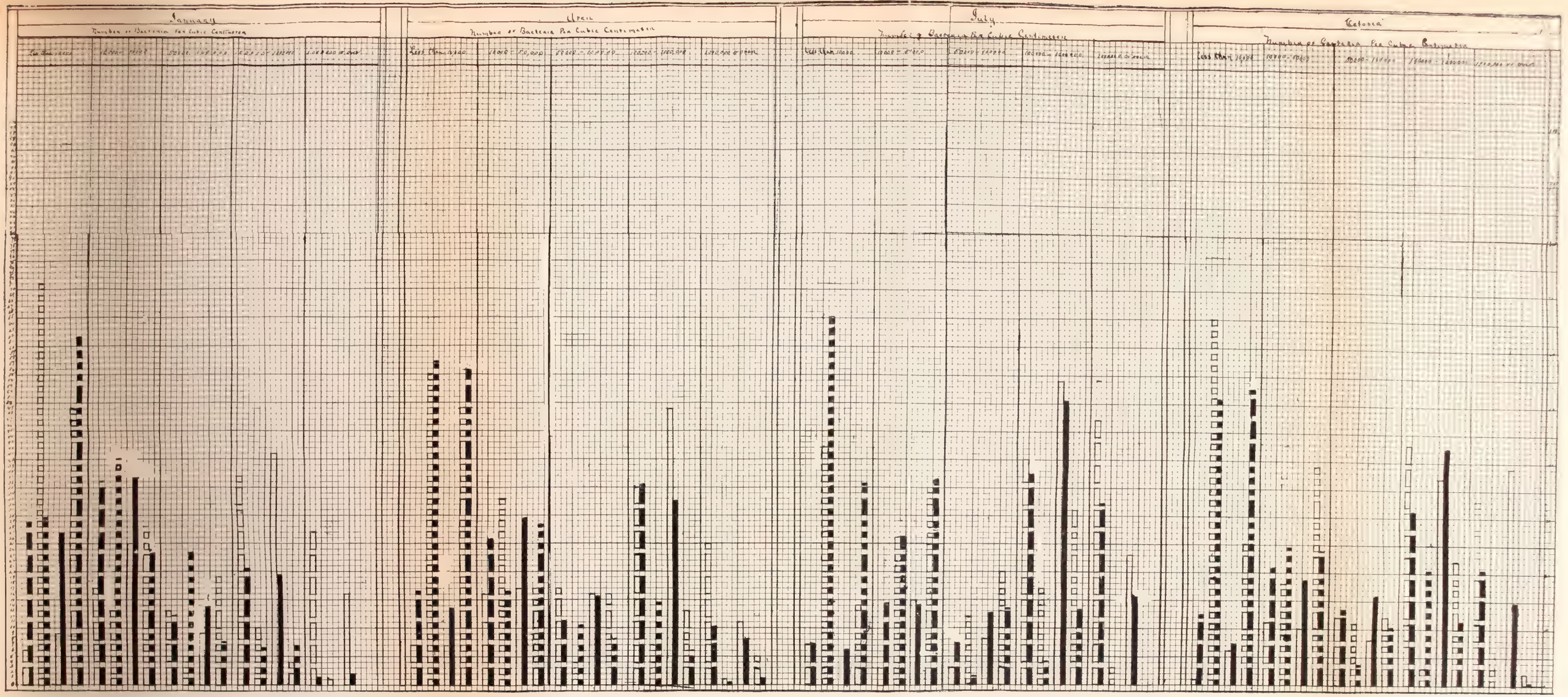
TABLE 2

SUMMARY OF BACTERIOLOGICAL COUNTS FROM PASTEURIZATION PLANTS

	Total No. of Samples	Samples from Mixing Vat — Bacteria Per Cubic Centimeter														Index No.	Samples from Holder — Bacteria Per Cubic Centimeter												Index No.	Samples from Bottles for Shipment — Bacteria Per Cubic Centimeter													
		Less than 10,000		10,000 to 50,000		50,000 to 100,000		100,000 to 250,000		250,000 to 500,000		500,000 to 1,000,000		1,000,000 to 5,000,000			Less than 500		500 to 1,000		1,000 to 5,000		5,000 to 10,000		10,000 to 50,000		50,000 to 100,000		100,000 to 250,000			Less than 500		500 to 1,000		1,000 to 5,000		5,000 to 10,000		10,000 to 50,000		50,000 to 100,000		100,000 to 250,000		250,000 to 500,000		500,000 to 1,000,000		Over 1,000,000	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
January	24	3	12 5	11	45 8	6	25	4	16 6							24	16	66 6	2	8 3	6	25								21	7	33 3	6	28 6	1	4 8	1	4 8										
February	25	3	12	9	36	2	8	5	20	1	4	3	12			29	8	27 5	8	27 5	9	31								19	5	26 3	7	36 8	2	10 5	1	5 3										
March	38	2	5 2	7	18 4	5	13 1	11	28 9	1	2 6	3	7 8	1	2 6	17	7	41 1	1	2 3	4	23 5	2	11 7						24	5	20 8	8	33 3	3	12 5	1	4 2										
April	52	5	9 6	20	38 4	7	13 4	13	25	6	11 5	1	1 9			11	15	60 9	1	9	11	26 4	1	2 4	3	10 3				31	9	29	9	29	4	12 9												
May	40			4	10	10	25	14	35	4	10	1	2 5			15	11	73 3	1	6 7	3	20	1	6 7						19	3	15 8	8	42 1	3	15 8	1	5 3										
June	16			1	6 2	1	6 2	3	18 7	1	6 2	1	6 2	1	6 2	11	7	63 6	1	9 1	2	18 2	1	9 1						10	2	20	4	40	1	10	1	10										
July	28	1	3 5	1	14 3	3	10 7	3	10 7	1	3 5	2	7 1	1	3 5	10	1	10	1	10	1	10	1	10	1	10				11	2	18 2	4	36 4	1	9 1	1	9 1	1	9 1								
August	5							1	20			3	60			9	2	22 2	1	11 1	2	22 2	1	11 1	1	11 1				9	1	11 1	3	33 3	1	11 1	1	11 1	1	11 1								
September	5							1	20			4	80			1	1	20			1	20	1	20	1	20				9	1	11 1	3	33 3	1	11 1	1	11 1	1	11 1								
October	5	1	20	1	20	1	20	1	20							8	3	37 5	2	25	2	25	2	25						11	4	36 4	4	36 4	1	9 1	1	9 1										
November	12			1	8 3	1	8 3			1	8 3	1	8 3	1	8 3	12	3	25	1	8 3	3	25	4	33 3						13	3	23 1	1	7 7	1	7 7	1	7 7	1	7 7	1	7 7	1	7 7				
December	6	1	16 6	2	33 3	1	16 6	2	33 3							3	1	33 3			1	33 3			1	33 3				2	1	50														
Total	251	16	6 3	59	23 5	37	14 7	62	24 7	17	10 7	20	7 9	23	9 1	7	2 7	222	81	37 8	47	16 6	51	22 9	19	8 5	14	6 3	5	4	1 8	253	57	20 5	24	9 4	26	10 3	31	12 3	16	6 3	12	4 7	3	1 1	2	7		

CHART II.

GRAPHIC PRESENTATION OF BACTERIAL CONTENT CITY MILK, 1911-1912.



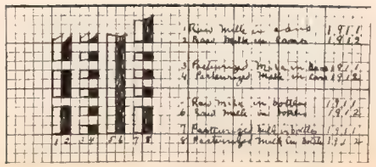


Chart No. II.

A STUDY OF THE AGGLUTINATION AND COMPLEMENT FIXATION REACTIONS IN GLANDERS AS COMPARED WITH CLINICAL SYMPTOMS AND AUTOPSY FINDINGS

Bertha van Houten Anthony and Marie Grund

During the past year the following work was carried out in order to determine the relative values of the sero-diagnostic reactions in glanders, especially when confirmed by clinical symptoms and autopsy findings. Since the two serum reactions depend upon entirely different substances in the blood it cannot be expected that the two methods should tally in every instance. It would seem that the only way in which definite conclusions can be reached is by continued study of all the data possible in long series of cases.

K. F. Meyers[1] has reported 210 cases, giving the sero-diagnostic reactions together with eye mallein and autopsy results. He found that the agglutination reaction occurred in only 74.5 per cent. of the positive cases, while the complement fixation test picked up 99.6 per cent.

In our own series 822 horses were tested, but the lack of data on 222 horses reduces the number to 600 from which deductions can be made. These fall into three groups:

(1) Autopsy (3 cases were not glandered)[2], Table I..... 76 cases
(2) Destroyed for glanders, Table II..... 119 cases
(3) Apparently normal[3] ..... 405 cases

Total..... 600 cases

The macroscopic agglutination test used was the incubator method.[4] The tubes, containing the stock test fluid and serum in dilutions of 1-500, 1-800, 1-1000 were incubated usually for three days at 37° C. Strongly positive reactions could be read often at the end of twenty-four to forty-eight hours. In the case of indeterminate reactions it was found later in the work that they would often clear up on further incubation even up to seven or ten days.[5] Negative controls in this additional incubation did not become positive even after a period of nineteen days in the incubator.

The complement fixation tests were carried on by the staff of Dr. Archibald McNeil, of the Research Laboratory of the New York Department of Health.

In comparing the agglutination and complement fixation reactions on 600 horses not all of the tests agreed with the histories.

In 193 *positive cases:*

The agglutination method (macroscopic) picked up 164 (about 84 per cent.), while the complement fixation method picked up 171 of the cases (about 90 per cent.).[6]

[1]Jour. Inf. Dis., 1913, XII, 170.
[2](A 460, A 134, 525.)
[3]Even here in some cases the marked discrepancies between the clinical symptoms and tests as compared with the final report of "normal" or "no case" cast doubt on the completed history.
[4]Jour. Infect. Dis., 1907, Supp. No. 3, p. 85.
[5]Dr. D. J. Mangan made this suggestion.
[6]We are indebted to Dr. R. H. Kingston for many of the histories of these cases, and data on eye mallein reactions.

The agglutination was negative in 11 cases; in which the complement fixation was positive in 8 cases, doubtful in 2, negative in 1.

The complement fixation was negative in 11 cases; in which agglutination had 7 positive, 3 doubtful, 1 negative.

The one case (A 430, Table I.), in which both reactions failed was caught by the eye mallein test, which gave a strong reaction in less than six days after the blood test and a prompt autopsy showed lung lesions. The importance of the mallein in this case is readily apparent.

In considering the doubtful or indefinite reactions:

The agglutination was doubtful in 18; complement fixation having 12 positive; 4 negative, 2 doubtful.

Complement fixation was doubtful in 10; agglutination having 7 positive, 2 negative, 1 doubtful.

In 405 *apparently normal horses:*

The agglutination gave 20 false positives; while the complement fixation had 7 positives, 2 doubtfuls, 11 negatives.

The complement fixation gave 20 false positives; while agglutination had 8 doubtful, 12 negative.

Thus 7 normal or supposedly normal horses were diagnosed as glandered by both tests. The mallein was negative in 4, positive in 1, yet it was returned as a "no case"; data as to the other 2 are wanting.

The doubtful or indefinite reactions were as follows:

The agglutination was doubtful in 81; complement fixation 8 positive, 68 negative, 5 doubtful.

Complement fixation was doubtful in 21; agglutination had 2 positive, 19 negative.

Of the 81 suspicious or doubtful agglutinations, 40 were 1-500 reactions only. It would seem that this reaction should be considered a negative one were it not for the fact that occasionally it may indicate an early glanders infection; for example, in case A 433, Table I., where there was a 1-500 agglutination after seven days incubation of test, a negative complement fixation; the horse broke down in two days, showing extensive lung lesions at autopsy. It is evident that neither the agglutination nor the complement fixation test should be relied upon to the exclusion of the other.

Microscopic method

Since the microscopic method had been used for a time in the New York Department of Health as a guide to the use of the mallein test, 90 *cases* were tested by the three methods, macroscopic agglutination, microscopic agglutination and complement fixation, which agreed with one another and with the diagnosis and outcome in 44 cases.

Of the disagreeing tests in 30 *normal horses:*

Microscopic was positive in 18; of these macroscopic gave 4 positives, 9 negatives, 5 doubtful reactions; complement fixation was positive in 7, doubtful in 5, negative in 6.

In 60 glandered horses:

Microscopic was positive in 43; macroscopic—positive 39, doubtful 3, negative 1.

Microscopic was doubtful in 11; macroscopic—positive 10, negative 1, while complement fixation was positive in these cases.

Microscopic was negative in 6; macroscopic—positive 2, doubtful 2, negative 2, while complement fixation was doubtful in 2, positive in 4.

Macroscopic was positive in 51; microscopic—positive in 39, doubtful in 10, negative in 2.

Macroscopic was doubtful in 5; microscopic—positive in 3, negative in 2.

Macroscopic was negative in 4; microscopic—positive in 1, doubtful in 1, negative in 2.

The complement fixation was negative in 4; microscopic—positive in 4.

The complement fixation was doubtful in 5; microscopic—positive in 3, negative in 2.

The complement fixation was positive in 16; microscopic-doubtful in 12, negative in 4.

CENTRIFUGE AGGLUTINATION METHOD

In order to compare the incubator and centrifuge methods[7] of macroscopic agglutination 74 tests were done in duplicate. Below are given the results in comparison with the complement fixation test of the same specimens of blood.

The time factor is greatly reduced by the centrifuge method. With it the tests are incubated for one hour, then centrifugalized for ten minutes. After two hours at room temperature the stronger reactions may be read, the others next day.

In 74 cases the three tests agreed with one another and with the clinical history and outcome in 40 cases.

Disagreeing reactions in 28 normal horses:

Incubator method gave 2 false positives; the centrifuge 1 positive, 1 doubtful; complement fixation gave 1 positive, 1 negative.

Incubator method gave 6 doubtfuls; the centrifuge 4 doubtfuls, 2 negatives.

Centrifuge method gave 1 positive; complement fixation gave 1 positive.

Centrifuge method gave 5 doubtfuls; complement fixation gave 4 positive, 1 negative.

Centrifuge method gave 2 negative; complement fixation 2 negative.

48 *glandered horses:*

Incubator method gave 10 doubtfuls; centrifuge 8 doubtful, 1 negative, 1 positive.

Centrifuge method gave 3 negatives; incubator 2 negative, 1 doubtful.

Complement fixation gave 16 positive; centrifuge 13 doubtful, 3 negative.

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[7]Mohler and Eichhorn—Bureau of Animal Industry—Circular No. 191.

Complement fixation gave 3 negative; centrifuge 1 positive, 2 doubtful.
Complement fixation gave 1 doubtful; centrifuge 1 positive.

Among the 74 cases there were 10 in which it was impossible to read the centrifuge reactions, while those of the incubator method were readable in all but 1. This one occurred before the prolonged method of incubation was used.

In 17 cases all four tests were also applied to the same specimens of blood. The results are given in Table III.

The prolonged method of incubation up to ten days has been tried and with it 14 glandered horses which gave weak, doubtful or indeterminate reactions were cleared up and became positive. In 19 normal horses indeterminate reactions became good negatives; making 33 improved reactions. In 24 other clinically normal horses the reactions became suspicious or positive. While this would seem a disadvantage, the results with other sera have been so satisfactory that this method will be carried on and a further report made later.

Vaccinated Horses.—While studying the effect of immunizing injections of glanders vaccine, Mohler and Eichhorn[8] reported that the agglutination began to show about the third day and persisted for six weeks to two months; that the complement fixation reaction appeared on the seventh to the ninth day and lasted for two or three months after the last vaccination. In a preliminary report no increased resistance to glanders was found in a test set of horses artifically exposed after vaccination.

Over 30 horses have been vaccinated at various times since the 8 given in Table IV. Three of these horses developed glanders, Table V., one within a month after vaccination and was destroyed on the clinical symptoms. The other two showed glanders lesions at autopsy, one three and the other four months after vaccination. All had given positive mallein and sero-diagnostic reactions before the vaccine. It is not possible to make any deductions as to the results on the remaining horses, as the time has not been long enough.

It is especially difficult to judge whether there has been any protection by vaccine in a horse under ordinary working conditions. Owners who have horses which give positive sero-diagnostic reactions and show no clinical symptoms, not only guard these horses from further infection, but are very careful not to overwork them. Thus good care, feeding and rest may combine to arrest the disease independently of vaccine. Table IV. shows the persistence of the effects of vaccine on the sero-diagnostic tests.

Mallein.—Eight apparently normal horses were tested before the subcutaneous administration of mallein and retested at varying intervals, as shown in Table IV. Within a week there was no change from the mallein. After a three-week interval its effect was apparent in all but one horse in the complement fixation and in all but two other horses which gave only suspicious agglutination reactions. By the seventh week its effects had

[8]Am. Vet. Review, 1913, XLIV.

begun to wane and in four months all the horses were negative except one which persisted in giving a 2 plus complement fixation test.

Eye Mallein.—As far as this has been used it would seem to be a method superior to the cumbersome subcutaneous one and quite as reliable if not more so on account of the difficulties in taking frequent temperatures over a period of several days. It has proved to be all that K. F. Meyers[9] has claimed for it, and with a little careful observation on the part of those using it, it should soon supplant the older method.

Discussion.—The results on 600 horses showed that neither the agglutination nor the complement fixation picked up all the cases, although only one glanders case was missed by both tests (A 430). Both tests pronounced 7 supposedly normal horses as glandered. The weakness of the agglutination test lies in its larger number of doubtful reactions in comparison with the complement fixation and the longer period required for diagnosis, especially if the prolonged method of incubation is used.

That neither the agglutination nor the complement fixation test should be used to the exclusion of the other is evident, also that mallein is very necessary to check up doubtful or even some negative cases. Eye mallein being easily applied makes the mallein test far more practicable than formerly. It should be used extensively to exclude all chance of error.

The prolonged period of incubation seems to increase the efficiency of the agglutination test in clearing up doubtful or indeterminate reactions and making weak positives stronger. That some indeterminate reactions were made suspicious or even positive in apparently normal horses is not so bad as may seem. A suspicious or positive reaction calls only for the use of eye mallein and subsequent re-testing. The additional labor is nothing in comparison with missing a source of glanders infection.

On 222 horses no record could be obtained. If glanders is to be stamped out a closer supervision of horses giving doubtful or suspicious reactions is imperative. A method by which horses can be permanently marked so they can be registered by the city is much needed. The changing of such marks should be penalized. The location of all stables, especially boarding-stables, should be recorded and when glanders appears such stables should be carefully watched, and the horses tested at frequent intervals.

The glanders question can never be intelligently handled until careful records are kept and a thorough system of co-operation among owners, veterinarians and the laboratory is established.

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[9]Loc. cit.

TABLE I

Seventy-Six Autopsy Horses

Case	Clinical Symptoms	Agglutination	Complement Fixation	Mallein	Remarks	Autopsy
239	.....	Pos.	Pos	+	Mallein and Vaccine† before blood test.	Glanders lesions.
283	.....	Pos.	Neg.	±	Vaccine 4 mos. before blood test. Mallein 1 mo. before blood test.	" "
285	Slight.....	Pos.	Pos.	.....	Vaccine 2 mos. before blood test.	" "
287	.....	Pos.	Pos.	.....	Vaccine 1 week before blood test.	" "
292	.....	Pos.	Pos.	+	Vaccine 3 or 4 weeks before blood test. Mallein few days before blood test.	" "
293	Nasal lesions.....	Pos.	Pos.	.....	.....	" "
314	None.....	Pos.	Pos.	+	Broke down with glanders later.	" "
360	Skin lesions.....	Pos.	Pos.	.....	.....	" "
361	" "	Pos.	Pos.	.....	.....	" "
362	" "	Pos.	Pos.	.....	.....	" "
368	Farcy buds on hock.....	Pos.	Pos.	+	.....	" "
393	Suspicious.....	Pos.	Pos.	.....	Killed 9 days after blood test	Lungs—slight lesions. Bronchial glands—slight lesions.
404	Clinical glanders.....	Pos.	Pos.	.....	.....	Glanders lesions.
405	" "	Pos	Pos.	.....	.....	" "
408	.....	Susp.	Doubt.	.....	.....	Lung lesions.
413	Persistent fluctuating fever.....	Pos.	Pos.	+	Mallein 16 days before blood test.	Lungs—extensive lesions. Lymph nodes—lesions.
417	.....	Pos.	A. C.	.....	.....	Lungs—extensive lesions.
418	Clinical glanders.....	Pos.	Pos.	.....	.....	" " "
426	Fever; suspected glanders.....	Pos.	Neg.	+	Mallein 6 days before blood test.	Lungs—numerous foci—acute and chronic.
463	Loss flesh—temperature 102° F. Suspected glanders.....	Pos.	Pos.	+	.....	Lungs—numerous foci.
475	Fluctuating temperature.....	Pos.	Doubt	+	Mallein just after blood test	Lungs—numerous foci.
477	Exposed.....	Pos.	Doubt.	+	.....	Lungs—glanders lesions.
479	Enlarged submaxillary gland; nasal lesions—temperature 101.3° F.....	Susp.	Doubt.	++	Killed 25 days after blood test.	Lungs—glanders lesions. Submaxillary gland—glanders lesions.

492	Exposed	Pos.	Pos.	++	.....	Lung lesions.
499	Exposed; suspicious fever	Pos.	Pos.	+	Mallein just after blood test.	Lungs—marked lesions. Spleen—slight lesions.
500	Exposed; suspicious fever	Pos.	Pos.	+	Mallein just after blood test.	Lungs—marked lesions. Spleen—slight lesions.
510	Several farcy buds	Pos.	Pos.	+	Mallein 18 days before blood test.	Lungs—numerous foci. Bronchial glands—numerous foci.
524	Exposed—temperature 103° F	Pos.	Pos.	+	Developed clinical symptoms.	Lungs—slight lesions. Lymph nodes—slight lesions. Liver—glanders foci.
525	Exposed—temperature 100° F	Pos.	Pos.	+	.....	No positive lesions.
533	Skin lesions	Pos.	Doubt.	.....	.....	Lungs—extensive lesions.
562	Suspicious	Pos.	Pos.	.....	.....	Lungs—moderate lesions. Bronchial glands—slight lesions.
581	Suspicious; fever; loss of flesh	Pos.	Pos.	0	Killed 13 days later.	Lungs—extensive lesions.
809	Exposed to horse in next stall	Pos.	Pos.	.....	Killed 2 weeks later.	Lungs—extensive lesions. Bronchial glands—slight lesions.
881	.....	Neg.	Pos.	+	Six days after blood test slight enlargement of submaxillary gland. Two mos. afterbleeding =pos. mallein	Forty days after blood test. Lungs—extensive lesions.
884	.....	Pos.	Pos.	+	Mallein 29 days after blood test. Killed 3 days later.	Lungs—slight lesions. Bronchial glands—slight lesions.
897	.....	Pos.	Pos.	+	Positive agglutination—and mallein several months before this test.	Lungs—moderate lesions. Killed 2 months later. Liver—moderate lesions.
A6	Exposed	Neg.	Pos.	+	Mallein 1 mo. after blood test. Killed 5 days later.	Lungs—moderate lesions. Bronchial glands—moderate lesions.
A11	Exposed	Pos.	Pos.	+	Mallein 1 mo. after blood test. Killed 5 days later.	Lungs—moderate lesions. Bronchial gland—moderate lesions.
A21	Exposed	Pos.	Pos.	+	Mallein 1 mo. after blood test. Killed 5 days later.	Lungs—moderate lesions. Bronchial gland moderate lesions.
A29	Exposed	Pos.	Pos.	+	Mallein 1 mo. after blood test. Killed 5 days later.	Lungs—moderate lesions. Bronichal glands—moderate lesions.
A41	Exposed	Pos.	Pos.	+	Mallein 25 days after blood test. Killed 11 days later.	Lungs—moderate lesions. Bronchial gland—moderate lesions.
A49	Exposed	Neg.	Pos.	+	Mallein 25 days after blood test.	Lungs—extensive lesions.
A96	Enlarged submaxillary gland—clinical glanders	Pos.	Pos.	.....	Killed 2 days after blood taken.	Lungs—extensive lesions.
A134	Papular condition of nasal mucous membrane	Susp.	Pos.	−	Had given negative complement fixation and mallein reaction before.	Lungs—negative. Trachea—abscess.

TABLE I.—*Continued*

Case	Clinical Symptoms	Agglutination	Complement Fixation	Mallein	Remarks	Autopsy
A183	None	Pos.	Pos.	+	Mallein reaction 37 days after blood test. Killed next day.	Lungs—slight lesions. Bronchial glands—slight lesions.
A184	None	Pos.	Neg.	+	Mallein reaction 37 days after blood test. Killed next day.	Lungs—moderate lesions.
A185	None	Pos.	Pos.	+	Mallein reaction 37 days after blood test. Killed next day.	Lungs—slight lesions.
A186	None	Pos.	Pos.	+	Mallein reaction 37 days after blood test. Killed next day.	Lungs—moderate lesions. Bronchial glands—slight lesions.
A188	None	Pos.	Pos.	+	Mallein reaction 37 days after blood test. Killed next day.	Bronchial glands—slight lesions. Lungs—moderate lesions.
A190	None	Pos.	Pos.	−	Mallein reaction 37 days after blood test. Killed next day.	Bronchial glands—slight lesions. Lungs—moderate lesions.
A191	None	Pos.	Pos.	+	Mallein reaction 37 days after blood test. Killed next day.	Lungs—moderate lesions.
A194		Pos.	Pos.	.....	Killed 7 days after blood test.	Lungs—moderate lesions. Pleurisy with effusion.
A195		Pos.	Pos.	.....	Killed 7 days after blood test.	Lungs—moderate lesions.
A197		Pos.	Pos.	.....	Killed 7 days after blood test.	Lungs—extensive lesions—pleurisy.
A198		Pos.	Pos.	.....	Killed 7 days after blood test.	Lungs—extensive lesions.
A227	Not marked	Pos.	Pos.	.....	Killed 6 days after blood test.	Lungs and bronchial glands—moderate lesions.
A229		Neg.	Doubt	.....	Broke down 10 days after blood test. Hindlegs swollen—farcy buds. Killed 1 week later.	Lungs—moderate lesions.
A232	None	Pos.	Pos.	.....	No clinical symptoms at autopsy 1 week later.	Lungs—moderate lesions.
A233		Pos.	Pos.	.....	Killed 10 days later.	Lungs—moderate lesions.
A277	None	Pos.	Pos	.....	Killed 8 days later.	Lungs—moderate lesions.

A311	No lesions but slight fever	Pos.	Pos.	.....	Killed 9 days later.	Lungs—moderate lesions.
A364	.....	Pos.	Pos.	+1*	Killed 3 mos. later.	Lungs—slight lesions. Liver—extensive lesions.
A370	Suspicious	Pos.	Pos.	.....	Killed 6 days later.	Lungs—extensive lesions. Bronchial glands—moderate sions
A374	Exposed	Pos.	Pos.	+3*	.....	Bronchial glands—slight lesions. Lungs—pleurisy with adhesions.
A389	Exposed	Pos.	Pos.	+3*	.....	Lungs and bronchial glands—extensive lesions.
A404	Exposed	Pos.	Pos.	+1*	.....	Lungs—moderate lesions. Bronchial glands—slight lesions.
A424	.....	Pos.	Pos.	+2*	Vaccine 2 years ago. Killed 13 days after bleeding.	Lung lesions—glanders.
A428	Enlarged submaxillary gland; nasal hemorrhage	Pos.	Doubt.	+1*	Killed 10 days later.	Lung lesions—glanders.
A430	.....	Neg.	Neg.	+3*	Killed 3 days later.	Lungs—glanders lesions.
A433	General debility	Susp.	Neg.	0	Broke down 2 days later—almost died; killed.	Lungs—Extensive lesions. Nasal lesions.
A452	Exposed	Pos.	Pos.	+2*	.....	Lungs and bronchial glands—slight lesions.
A460	Exposed	Pos.	Pos.	—*	Killed 1 month later.	No lesions found.
A532	.....	Pos.	Pos	.....	Killed 2 weeks after bleeding	Lung lesions—glanders.
A536	Exposed	Pos.	Pos.	+2*	Killed 2 days after bleeding.	Lungs—moderate lesions. Bronchial glands—extensive lesions.
A576	None	Pos.	Pos.	.....	Killed a few days later.	Lungs—moderate lesions.
A582	None	Pos.	Pos.	+	Mallein 9 mos. before.	Bronchial glands—moderate lesions. Lungs—one nodule.

†As regards many of the vaccinations recorded in Tables I and II no data could be obtained other than the fact that vaccine had been given.

*Eye Mallein.

TABLE II

One Hundred and Nineteen Horses Destroyed for Glanders

Case	Clinical Symptoms	Agglutination	Complement Fixation	Mallein	Remarks	Outcome
248	None	Pos.	A. C.	.....	In badly infected stable.	Destroyed.
249	None	Pos.	A. C.	.....	In badly infected stable.	Destroyed.
266	Skin lesions	Susp.	Pos.	?	.....	Destroyed.
267	Skin lesions	Neg.	Pos.	?	.....	Destroyed.
268	Skin lesions	Pos.	Pos.	?	.....	Destroyed.
269	Skin lesions	Pos.	Pos.	?	.....	Destroyed.
270	Skin lesions	Pos.	Pos.	?	.....	Destroyed.
271	Skin lesions	Pos.	Pos.	?	.....	Destroyed.
275	Skin lesions	Pos.	Pos.	?	.....	Destroyed.
279	None	Susp.	Pos.	.....	.....	Destroyed 1 month later.
280	Glanders	Pos.	Pos.	.....	.....	Destroyed day of bleeding.
282	Suspicious	Neg.	Pos.	.....	.....	Destroyed.
286	Ulcers and farcy buds	Pos.	Pos.	.....	.....	Destroyed.
288	Nasal ulcers and farcy buds	Pos.	Pos.	0	.....	Destroyed.
289	Glanders	Pos.	Pos.	0	.....	Destroyed.
317	Suspicious buds (?)	?	Pos.	0	.....	Destroyed.
350	Swollen fore and hind leg—chronic lymphangitis—enlarged submaxillary gland	Pos.	Pos.	0	.....	Destroyed 1 week later.
351	Enlarged submaxillary gland	?	Pos.	.....	.....	Destroyed about 1 month later.
353	Suspicious	Pos.	Pos.	.....	Clinical glanders developed.	Died.
357	None	Neg.	Doubt.	+	Mallein positive reaction 5 days later.	Destroyed.
358	None	Susp.	Pos.	+	Mallein positive reaction 5 days later.	Destroyed.
359	None	Neg.	Pos.	+	Mallein reaction 5 days later.	Destroyed.
379	Glanders	Pos,	Pos.	.....	.....	Destroyed day of bleeding.
380	Suspicious	Pos.	Pos.	.....	Vaccine 3 weeks before.	Destroyed day of bleeding.
381	.....	Pos.	Neg.	.....	.....	Destroyed.
382	Farcy buds on hind leg	Pos.	Pos.	.....	.....	Destroyed 11 days later.
384	Glanders	Pos.	Pos.	0	.....	Destroyed.
385	Glanders	Pos.	Pos.	0	.....	Destroyed.
386	Glanders	Pos.	Pos.	0	.....	Died of glanders.
392	Suspicious—constant fever (102²/₅° F.)	Pos.	Neg.	±	.....	Destroyed.
395	.....	Pos.	Pos.	+	.....	Died of glanders 3 days later.
397	Skin lesions	Pos.	Pos.	.....	.....	Destroyed.
398	Skin lesions	Pos.	Pos.	.....	.....	Destroyed.
399	Skin lesions	Pos.	Pos.	.....	.....	Destroyed.

400	Skin lesions	Pos.	Pos.	.....	.....	Destroyed.
401	Skin lesions	Pos.	Pos.	.....	.....	Destroyed.
402	Skin lesions	Pos.	Pos.	.....	.....	Destroyed.
406	.....	Pos.	Pos.	.....	.....	Destroyed 10 days later.
407	Enlarged submaxillary gland and nasal discharge	Pos.	Pos.	.....	.....	Destroyed 4 days later.
416	Glanders	Pos.	Pos.	.....	.....	Destroyed.
419	Farcy skin lesions	Pos.	Pos.	.....	.....	Destroyed.
420	Farcy skin lesions	Pos.	Pos.	.....	.....	Destroyed.
421	Farcy skin lesions	Pos.	Pos.	.....	.....	Destroyed.
422	Farcy skin lesions	Pos.	Pos.	.....	.....	Destroyed.
423	Farcy skin lesions	Pos.	Pos.	.....	.....	Destroyed.
424	Farcy skin lesions	Pos.	Pos.	.....	.....	Destroyed.
425	Temperature 101° F.—Enlarged glands	Pos.	Pos.	.....	.....	Destroyed.
427	Swollen hind leg—suspicious purulent lesions —temperature 101° F.	Pos.	Pos.	.....	.....	Destroyed.
459	Nasal discharge—Enlarged submax. gland	Pos.	Pos.	.....	.....	Destroyed next day.
460	Submaxillary gland enlarged—Abscesses	Pos.	Pos.	.....	.....	Destroyed 2 days later.
461	.....	Pos.	Pos.	.....	.....	Died of glanders 1 week later.
462	Exposed	Susp.	Pos.	0	.....	Destroyed.
464	Old scar with new buds on hock	Pos.	Pos.	.....	.....	Destroyed 5 days later.
465	Exposed—temperature 102° F.	Pos.	Pos.	.....	.....	Destroyed 5 days later.
466	Suspicious—temperature 103° F.	Pos.	Pos.	.....	.....	Destroyed 3 weeks later.
467	Exposed—temperature 100° F.	Pos.	Pos.	.....	.....	Destroyed 1 month later.
470	Glands enlarged—temperature 102° F.	Pos.	Pos.	.....	.....	Destroyed 5 days later.
473	Enlarged submaxillary gland	Pos.	Pos.	.....	.....	Destroyed 1 month later.
496	Suspicious	Pos.	Pos.	.....	.....	Destroyed next day.
506	Suspicious	Susp.	Pos.	+	Mallein reaction 10 days after blood test.	Destroyed 2 weeks later.
509	.....	Pos.	Pos.	.....	.....	Destroyed 8 days later.
535	.....	Pos.	Pos.	.....	Farcy buds developed.	Destroyed 9 days later.
563	Swollen off hind leg. (Abscesses)	Pos.	Pos.	.....	.....	Destroyed 16 days later.
564	Suspicious	Pos.	Pos.	.....	Farcy buds developed.	Destroyed.
565	Suspicious—sick 1 month—Temp. 102° F.	Pos.	Pos.	.....	Vaccine 5 weeks after blood test. Farcy buds developed.	Destroyed 6 weeks after bleeding
567	Suspicious	Susp.	Pos.	.....	.....	Destroyed 1 week later.
568	Suspicious	Pos.	Pos.	+	.....	Destroyed 1 week later.
569	Buds on hind leg of short duration	Pos.	Pos.	+	.....	Destroyed.
571	None	Pos.	Pos.	0	.....	Died in stall of glanders 2 weeks later.
572	Glanders	Pos.	Pos.	.....	.....	Destroyed.
573	Enlarged submaxillary gland—nasal discharge—wound on leg.	Pos.	Pos.	0	.....	Destroyed.
583	Glanders	Pos.	Pos.	.....	.....	Destroyed a few days later.
584	None	Pos.	Pos.	.....	.....	Destroyed 11 days later.
586	Suspected glanders—lymphangitis	Pos.	Pos.	+	.....	Destroyed 3 days later.
588	Exposed	Susp.	Pos.	+	No clinical symptoms.	Destroyed 1 week later.
594	Exposed	Susp.	Pos.	+	No clinical symptoms.	Destroyed 1 week later.

TABLE II.—*Continued*

Case	Clinical Symptoms	Agglutination	Complement Fixation	Mallein	Remarks	Outcome
596	Exposed	Neg.	Pos.	+	.....	Destroyed 2 weeks later.
599	Exposed	Susp.	Pos.	+	.....	Destroyed 1 week later.
609	Exposed	Neg.	Pos.	?	No clinical symptoms.	Destroyed 2 weeks later.
616	Glanders	Pos.	Pos.	0	.....	Destroyed.
617	Glanders	Pos.	Pos.	0	.....	Destroyed.
618	Glanders	Susp.	Neg.	0	.....	Destroyed.
619	Glanders	Pos.	Pos.	0	.....	Destroyed.
620	Glanders	Pos.	Pos.	0	.....	Destroyed.
621	Glanders	Pos.	Pos.	0	.....	Destroyed.
622	Glanders	Pos.	Pos.	0	.....	Destroyed.
623	Glanders	Pos.	Pos.	0	.....	Destroyed.
624	Glanders	Pos.	Pos.	0	.....	Destroyed.
625	Glanders	Pos.	Pos.	0	.....	Destroyed.
626	Glanders	Susp.	Neg.	0	.....	Destroyed.
627	Glanders	Pos.	Pos.	0	.....	Destroyed.
628	Glanders	Pos.	Pos.	0	.....	Destroyed.
629	Glanders	Pos.	Pos.	0	.....	Destroyed.
721	Submaxillary glands enlarged—Farcy buds	Pos.	Pos.	.....	Vaccine about 1 year before.	Destroyed.
862	Poor condition 1 month	Susp.	Pos.	.....	.....	Destroyed 2 months later.
A98	.....	Pos.	Pos.	.....	Large dose vaccine (5 c. c.) on day of bleeding.	Died early next morning.
A99	Probably glanders—temperature 103° F.	Pos.	Pos.	.....	Large swelling—after 5 c. c. vaccine on day of bleeding.	Destroyed 2 days later.
A101	.....	Pos.	Pos.	.....	Large painful swelling—after 5 c. c. vaccine on day of bleeding—Nasal Chancres.	Destroyed 2 days later.
A100	.....	Pos.	Pos.	.....	Developed glanders symptoms after 5 c. c. vaccine on day of bleeding.	Destroyed 6 weeks later.
A149	None	Pos.	Pos.	+*	.....	Destroyed 3 months later.
A160	.....	Pos.	Pos.	.....	.....	Died of glanders.
A162	.....	Pos.	Pos.	.....	.....	Died of glanders.
A164	.....	Pos.	Pos.	.....	.....	Destroyed 7 days later.
A166	Enlarged submaxillary gland	Pos.	Pos.	.....	Nasal chancers.	Destroyed 4 days later.
A182	.....	Pos.	Pos.	.....	Clinical glanders—skin lesion developed.	Died 25 days after blood test.
A187	General condition good	Pos.	Neg.	.....	Glanders developed—extensive skin lesions.	Destroyed 25 days after blood test.
A189	General condition good	Pos.	Pos.	.....	Glanders developed—extensive skin lesions.	Destroyed 25 days after blood test.

A276	None	Pos.	Pos.	.....	.....	Died 8 days later—glanders.
A280	.....	Pos.	Pos.	*+[1]	.....	Destroyed.
A281	Intermittent fever	Pos.	Pos.	.....	Glanders symptoms developed.	Destroyed.
A315	Apparently fair condition	Pos.	Pos.	.....	.....	Died 2 days later.
A321	Farcy buds on legs	Pos.	Neg.	.....	.....	Destroyed on day of bleeding.
A337	.....	Pos.	Pos.	*+[1]	Vaccine about 1½ years ago.	Destroyed.
A363	None	Pos.	Pos.	0	Three days after bleeding nasal chancres and submaxillary gland developed.	Destroyed when almost dead.
A367	.....	Pos.	Pos.	+	Large swelling after vaccine.	Killed 1 month later, clinical glanders.
A368	Suspicious	Pos.	Pos.	.....	Clinical glanders developed.	Destroyed 4 days later.
A369	.....	Pos.	Pos.	.....	Clinical glanders developed.	Destroyed 4 days later.
A426	Suspicious	Pos.	Pos.	*+[2]	Skin lesions appeared.	Destroyed.
A427	Suspicious	Susp.	Pos.	*+[2]	Eye mallein 5 days later.	Died 8 days later of glanders.

*Eye Mallein.

Interpretation of reactions—

		1–500	1–800	1–1000
Macroscopic Agglutination:				
	Positive	++	++	++
		+	+	+
		+	+	±
	Suspicious	+	±	±
		+	±	−
		+	−	−
	Indeterminate (?)	±	±	±
		±	±	−
	Negative	±	−	−
		−	−	−

Microscopic Agglutination:

Positive =+ in 1–2000
+ in 1–1000
Suspicious =+ in 1–500
Negative =no reaction

Complement Fixation:

Positive =++++, +++, ++
Doubtful =+, ±
Negative =−
Anticomplementary =A. C.

Mallein—Unless otherwise stated the mallein results given refer to the subcutaneous method. + = positive; ± = slight reaction; − = negative; ? = questionable reaction; 0 = no mallein. Where the space is blank data as to whether or not mallein was given are wanting.

Eye Mallein— − = negative; sl. = slight; + = reaction; $+^2$ = one or both lids swollen; $+^3$ and $+^4$ = purulent discharge and eye closed and swollen.

TABLE III

SEVENTEEN CASES, FOUR COMPARISONS

Case	Clinical Symptoms	Agglutination			Complement Fixation	Mallein	Outcome
		Macroscopic		Microscopic			
		Incubator	Centrifuge				
477	Exposed	Pos.	Pos.	Pos.	Doubt.	+	Autopsy— L u n g s—glanders lesions.
478	Exposed	?	?	Pos.	Neg.	.....	Unknown.
479	Enlarged submaxillary gland—Nasal lesions	Susp.	Pos.	Pos.	Doubt.	+	Killed 25 days later. Autopsy—Lungs—glanders lesions. Submaxillary gland—glanders lesions.
480	Fever	Pos.	Pos.	Pos.	Doubt.	?	Unknown.
481	Exposed	Neg.	Neg.	Neg.	Neg.	.....	Unknown.
482	Exposed	Neg.	Neg.	Neg.	Neg.	.....	Unknown.
483	Exposed	Neg.	Neg.	Neg.	Neg.	.....	At work.
484	Exposed	Susp.	Neg.	Susp.	Neg.	.....	At work.
485	Exposed	Neg.	Neg.	Neg.	Neg.	.....	At work.
486	Exposed	Neg.	Neg.	Neg.	Neg.	.....	At work.
487	Exposed	Susp.	Neg.	Neg.	Neg.	.....	At work.
488	Exposed	Neg.	Neg.	Neg.	Neg.	.....	At work.
489	Exposed	Susp.	Pos.	Pos.	Neg.	.....	At work.
490	Exposed	Neg.	Neg.	Susp.	A. C.	.....	At work.
491	Exposed	Neg.	Neg.	Neg.	Pos.	.....	At work.
492	Exposed	Pos.	Pos.	Pos.	Pos.	+	Autopsy—Lung lesions.
493	Exposed	Neg.	?	Pos.	Neg.	.....	At work.
	Positive Reactions	3	5	7	2		3 Glanders Cases.
	Doubtful Reaction	5	2	2	4		10 Apparently Normal Cases.
	Negative Reactions	9	10	8	11		4 Unknown.

TABLE IV

EIGHT APPARENTLY NORMAL HORSES WHICH RECEIVED MALLEIN, THEN VACCINE

Horse	Date bled	Aggl 500 800 1000	Comp Fix	Mallein	Date bled	Aggl 500 800 1000	Comp Fix	Date bled	Aggl 500 800 1000	Comp Fix	Date bled	Aggl 500 800 1000	Comp Fix	Vaccine	Date bled	Aggl 500 800 1000	Comp. Fix	Vaccine	Date bled	Aggl 500 800 1000	Comp Fix	Vaccine	Clinical Symptoms	Date bled	Aggl 500 800 1000	Comp. Fix	Date bled	Aggl. 500 800 1000	Comp Fix	Date bled	Aggl. 500 800 1000	Comp Fix	Date bled	Aggl 500 800 1000	Comp Fix	Date bled	Aggl 500 800 1000	Comp Fix
4418	Bled August 31 1912 then received mallein	Neg	Doubt	Neg	September 3 1912	Neg	Doubt	September 19 1912	Pos	Pos	October 17, 1912	Susp	A. C	December 11, 1912	Neg.	Neg.	1 c c	December 18, 1912	Susp	Pos.	2½ c c	December 27, 1912	Pos	Pos	5 c c	January 4, 1913	Pos	Pos	10 c c		February 26, 1913	Neg.	Neg	March 6, 1913	Neg.	Neg	March 12, 1913	Neg.	Doubt	March 20, 1913	Neg.	Neg	April 17, 1913	Neg.	Neg
3850		Pos	Pos	Pos		Pos	Pos		Pos	Pos		Susp	Neg		Neg	Neg	1 c c			Neg	2½ c c		Pos	Pos	5 c c		Pos	Pos	10 c c	Large Sensitive Swelling		Pos	Pos		Susp	Doubt		Susp	Doubt.		Pos	Neg.		Neg	Neg.
4040		Neg.	Neg.	Neg		Neg.	Neg		Neg	Pos		Neg	Doubt		Neg	Pos	1 c c			Neg	2½ c c		Pos	Pos	5 c c		Pos	Pos	10 c c	Very Large Sensitive Swelling		Neg	Doubt		Neg	Pos		Neg	Neg		Neg	Neg		Neg	Neg
4381		Neg	Neg	Neg		Neg.	Doubt		Pos.	Pos		Neg	Doubt		Neg	Neg	1 c c				2½ c c		Pos	Pos	5 c c		Pos	Pos	10 c c	Large Sensitive Swelling		Susp	Pos		Susp	Pos		Susp	Pos		Pos	Pos		Susp	Doubt
4221		Susp	Doubt	Neg		Susp	Neg		Pos	Pos		Susp	Neg		Neg.	Neg	½ c c				1 c c		Pos	Pos	2½ c. c.		Pos	Pos	5 c c	Large Sensitive Swelling		Susp	Neg.		Neg	Neg		Neg	Neg		Susp	Neg.		Pos	Neg
4360		Pos	Neg	Neg		Pos	Neg		Pos	Neg		Susp	Neg		Neg	Neg.	½ c c			Neg	1 c c.		Pos	Pos	2½ c c		Pos	Pos	5 c c			Pos	Doubt		Pos	Doubt		Neg	Doubt		Pos	Doubt		Pos	Neg
4603		Neg	Doubt.	?		Neg.	Neg.		Pos	Pos		Neg	Neg		Neg.	Neg	½ c c			Doubt	1 c c		Pos	Pos	2½ c c		Pos	Pos	5 c c			Neg	Neg		Neg	Neg		Neg	Doubt		Neg	Neg		Neg	Neg
4351		Neg	Pos	Neg		Susp	Pos		Neg.	Pos		Neg	Neg		Neg	Neg.	½ c c				1 c c.		Pos	Pos	2½ c c		Pos	Pos	5 c c	Very Large Sensitive Swelling		Neg.	Neg		Neg	Neg		Neg	Neg		?	Neg		Neg	Neg

OUTCOME. December, 1913—All horses in good condition and working.

TABLE V

THREE VACCINATED HORSES

Horse	Date test	Aggl.	Comp. Fix.	Mallein	Vaccine	Remarks
	1913					
8025	June 16	Pos.	Pos.	....	...	Good condition; put in quarantine
	Aug. 8	Pos.	Pos.	+ eye	10c.c.	Large swelling.
	Aug 26	...	...	....	20c.c.	Large swelling.
	Sept. 13	...	...	....	...	Clinical glanders developed.
						Killed. No autopsy.
2179	June 6	Pos.	Pos.	....	...	
	Aug. 8	Pos.	Pos.	+ eye	10c.c.	Slight fever; large swelling.
	Aug. 26	...	...	....	20c.c.	Large swelling.
	Nov. 17	...	...	....	...	Killed. Autopsy: Lungs, slight lesions; liver, extensive lesions.
4	Feb. 14	...	...	+ subcutaneous	...	
	Apr. 28	Pos.	Pos.	....	...	
	May 4	...	...	....	2c.c.	
	May 11	...	...	....	5c.c.	
	May 18	...	...	....	10c.c.	
	July 27	Pos.	Pos.	....	...	
	Nov 9	...	...	....	...	Killed. Autopsy: Bronchial gland moderate lesions; lung, one nodule.

COMPARATIVE TESTS—EXAMINATION OF SPUTUM BY THE KINYOUN AND ELLIMAN-ERLANDSEN METHOD

Jane L. Berry and Mary A. Smeaton

In order to determine the comparative value of the Kinyoun and Elliman-Erlandsen methods for the examination of sputum, a series of tests were undertaken at Otisville during the summer of 1912.

Through the courtesy of the physicians at the Otisville Sanatorium, we were enabled to obtain samples of sputum from the sanatorium patients which we studied by the method recommended by Dr. Kinyoun, comparing our results with those obtained by the sanatorium physicians in their regular routine examinations, which are made by the Elliman-Erlandsen method.

The slightly modified Elliman-Erlandsen method in use at the sanatorium is as follows:

Sputum mixed with an equal volume of $NaCO_3$ solution.
Stir.
Incubate 24 hours at 37 degrees F.
Add 2-3 drops of 40 per cent. NaOH solution.
Boil.
Centrifuge.

As much as possible of the sediment is spread upon slides, in doubtful cases the entire sediment being examined.

By Dr. Kinyoun's method the sputum is mixed with 2-3 volumes of sodium hypochlorite of such dilution as to contain 56 per cent. of available chlorine. One cubic centimeter of ligroin is added next, the mixture thoroughly shaken until it has become homogeneous, then centrifuged, and examination made of the soapy layer lying directly below the ligroin, where the tubercle bacilli, if present, will be found to be concentrated. If the sputum is tenacious and not readily broken up, Dr. Kinyoun recommends allowing it to stand over night in the hypochlorite mixture after preliminary shaking. The shaking is repeated the next morning, after which the specimen is centrifuged and examined with either the ordinary carbol fuchsin stain or with the stronger stain recommended by Dr. Kinyoun.

Fuchsin basic (Grubler), 4 grams.
Acid carbolic g. p. crystal, 8 grams.
Alcohol 95 per cent., 20 cubic centimeters.
Water, 100 cubic centimeters.

Decolorization is done with 3 per cent. hydrochloric acid in 95 per cent. alcohol. Methylene blue is used as a counter stain.

With the exception of the use of the centrifuge, the above method was followed exactly in our work. We first examined a series of specimens, centrifuging one-half and allowing the other to stand at room temperature, after shaking, until the next morning. It was found that the tubercle bacilli, if present, would be found gathered together in the soapy layer without previous centrifuging, and since, in some cases, our results were less good in the centrifuged portions than in those which had simply stood over night, the latter method was adhered to in our further work.

With nearly all specimens direct smears were made as soon as the sputum was received, and these were compared with the smears from the hypochlorite and ligroin mixture.

The specimens were taken as they came in the ordinary course of the daily routine sputum examinations, and were from cases in all stages of pulmonary tuberculosis. All precautions as to solutions, sterile glassware and new slides were observed with both methods.

Comparison of Direct with Hypochlorite Method

By Direct Examination	*By Hypochlorite Method*
Positive, 118 .....72.4 per cent.	Positive, 135 .....82.8 per cent.
Negative, 45 .....27.6 per cent.	Negative, 28 .....17.2 per cent.

Became positive with hypochlorite, 20 ..... { 12.2 per cent. of whole number. / 44.4 per cent. of those negative by direct examination.

More strongly positive with hypochlorite, 34..... { 20.8 per cent. of whole number. / 28.8 per cent. of those positive by direct examination.

No decided advantage with hypochlorite, 79..... { 48.4 per cent. of whole number. / 66.9 per cent. of those positive by direct examination.

Comparison of Hypochlorite with Elliman-Erlandsen Method

Total number of specimens compared, 159.

Positive by Elliman-Erlandsen,
Negative by hypochlorite:

4 = 0.25 per cent. { 1 = many organisms. / 3 = few organisms.

Positive by hypochlorite,
Negative by Elliman-Erlandsen:

17 = 10.6 per cent. { 7 = many organisms. / 1 = moderate number. / 12 = few organisms.

Conclusions

By the use of the hypochlorite and ligroin method many specimens became positive which were negative by direct smear, and many others showed a greatly increased number of organisms.

In the comparison of the hypochlorite and ligroin with the Elliman-Erlandsen method, allowance must be made for differences of technique between two sets of people working in different places, and a longer search might account for some of the differences in results where but few organisms were found. Leaving these cases out of account, however, it is still evident that in this series of tests the advantage lies with the hypochlorite and not with the Elliman-Erlandsen method, since by the former there were seven strongly positive tests and one showing a moderate number of organisms all of which were negative by the other method, as compared with one strongly positive test by the Elliman-Erlandsen which was negative by the Kinyoun method.

TUBERCLE BACILLI IN THE BLOOD

Jane L. Berry

Notwithstanding the large number of investigators who have made a study of the presence of tubercle bacilli in the circulating blood, it is nevertheless a fact that the most striking point about the results so far obtained is the total lack of agreement between the reports of the different workers.

In a number of cases there is an absence of sufficient detail as to technique and controls, but leaving these records out of account there remain a long series of reports comprising the most careful and painstaking work described in minute detail, in which nevertheless the authors have come to absolutely contradictory conclusions.

A difference so marked could not fail to attract attention, and within the past year a number of extensive reviews of the literature have been made by different writers, especially by Bacmeister and Reuben, Elsaesser, and Rothacker and Charon.

As the majority of these articles are in German, and as far as known there is no corresponding record in English, a brief resumé of the principal articles is here given, including some of the most recent work on the subject.

The first investigator in this field seems to have been Villemin, who, in 1868, obtained positive inoculation results with the blood in miliary tuberculosis. Weichselbaum, in 1884, found tubercle bacilli microscopically in otherwise sterile blood from miliary tuberculosis. Next followed Meissel, Hildebrand, Huguenin, Sticker, Gary, Bergeron, Lüdke, Lustig, Rutimeyer and others who, either microscopically or by animal inoculation, demonstrated the presence of tubercle bacilli in the blood in a varying percentage of severe cases.

As a result of this work it became recognized that bacilli could be found in the blood irregularly in the last stages of miliary tuberculosis, with a possibility that the same might be true of certain severe pulmonary infections. The generally accepted opinion, however, continued to be that expressed by Bergeron in 1904, when he stated that tubercle bacilli are found in the blood in miliary tuberculosis, but only in relatively rare cases, and that in non-miliary tuberculosis, even of a rapid type, bacillaemia does not exist.

A greatly added impetus was given to the investigation by the introduction of the Stäubli acetic acid method in 1908, followed by the Schnitter modification in 1909. The Stäubli-Schnitter method is as follows: Ten to fifteen cubic centimeters of blood from the vein are added to a like amount of two to three per cent. citric acid or double the amount of three per cent. acetic acid, then carefully shaken and allowed to stand for one-half hour. Centrifuge and pipette off fluid, add one cubic centimeter water to sediment, then add two to five times the amount of fifteen per cent. antiformin and shake. As soon as solution is complete, which happens very quickly, the mixture is again centrifuged, the fluid pipetted off, and the slight deposit is washed and examined by the Ziehl-Nielson stain.

Schnitter found acid-fast bacilli in thirty-one and six-tenths per cent. of thirty-eight cases of tuberculosis. He found very few organisms, not more than five or six in most cases, and the majority of these were "granules." Schnitter came to the conclusion that tubercle bacilli are probably regularly present in the blood in organic tuberculosis, but that this does not necessarily lead to miliary infection, since the latter is due to a sudden explosive outburst of organisms into the blood in such numbers that the natural protective powers of the organism are unable to cope with them.

Just previous to the work of Schnitter, Rosenberger, in this country, had published his report of 100 per cent. positive microscopic results in forty-nine cases of tuberculosis in all stages, five miliary. Rosenberger drew blood into sterile sodium citrate, centrifuged and examined the sediment. In a later report Rosenberger brought his list of cases up to 300, in all of which he claimed to have found acid-fast bacilli in the blood.

Schroeder and Cotton, Burvill-Holmes, Anderson and other American workers attempted to repeat Rosenberger's work, using the same method, but utterly failed to reach the same conclusions. The English investigators, Hewat and Sutherland, working with cattle, also failed to corroborate Rosenberger, although Forsyth in England, with this method, reported 100 per cent. positive results in ten open cases of tuberculosis, negative results in two closed cases.

The work of Anderson was especially interesting on account of his inoculation and culture tests. All direct microscopic examinations of blood were negative, and of eighty-three guinea pigs inoculated intraperitoneally with human blood none developed tuberculosis, but the blood of thirteen tuberculous guinea pigs gave one positive result when injected into other guinea pigs, while of eight infected rabbits the blood of seven produced tuberculosis in guinea pigs. In three cases rabbit blood specimens gave cultures of tuberculosis, this being the first time, according to Anderson, that successful cultures have been reported from rabbits' blood.

With the finding by Brem, and afterward by others, of acid-fast bacilli in laboratory distilled water it was thought that a probable explanation could be given of the result reported by Rosenberger. Since then but little has appeared upon the subject in this country, but a large amount of work has been done in foreign laboratories, especially among the Germans.

Lippmann, with the Stäubli-Schnitter method, and new slides, found eleven (44 per cent.) positive in twenty-five pulmonary cases, all stages. Found very few bacilli and no connection between fever and positive blood test.

Jessen and Rabinowitsch, with a slightly modified Stäubli-Schnitter method, and all precautions as to slides, glassware, etc., had ten (28 per cent.) positive results in thirty-six cases, different stages. Ziehl, Much and Gram stains were used, and "granules" were included among positive results. The Ziehl stain gave the best results. No agreement was evident between the clinical stage and the presence of bacilli.

Acs-Nagy, with the Stäubli-Schnitter method, and careful aseptic

precautions, found eleven (45 per cent.) positive blood specimens in twenty-four cases, of which five were miliary, the remainder pulmonary tuberculosis of the second and third stages. The majority of organisms were "granules." No agreement was found between microscopic result and severity of case or time before death.

Kurashiga, with a modified Stäubli-Schnitter method and Ziehl stain, reported positive blood findings in the astonishing ratio of 100 per cent. in 155 cases, all stages. No details given as to asepsis and controls. Bacilli were said to be present in great numbers, as many as thirty in one field. Besides the above cases of pulmonary tuberculosis, Kurashiga examined the blood of thirty-four apparently healthy subjects and found in twenty (59 per cent.) acid-fast bacilli in the blood. In three of these cases tuberculosis developed during the next eight months. Guinea pigs inoculated with microscopically positive blood from four apparently normal persons developed tuberculosis. In a later paper Kurashiga gave the results of a series of repeated examinations of blood at intervals of one to two weeks. Of the twenty cases, all stages, from which the blood was taken, each gave positive results, and of the 114 specimens of blood 104 were positive. None were tested in animals. Kurashiga concludes that tuberculosis is primarily a bacillaemia, with later localization in the tissues and the appearance of characteristic symptoms.

Sturm, seeking to confirm Kurashiga's findings, came to quite different conclusions. With corresponding preparations, the one stained by the Much, the other by the Ziehl-Nielson method, he found by the former stain 42 per cent. and by the latter only 22 per cent. positive in fifty cases, all stages. Of ten specimens from apparently normal persons, all were negative. Animal tests, however, made by the inoculation of five to six c. c. blood intraperitoneally in guinea pigs gave the large number of 46 per cent. positive results. There was no agreement between the presence of bacilli in the sputum and in the blood, nor between the positive blood test and the severity of the case.

Krause-Hannover, using the Uhlenhuth antiformin method, had 11 per cent. positive results in thirty-two severe cases of the second and third stages. A second series of one hundred cases, all stages, gave 22 per cent. positive results. Many cases positive at one time were found negative at another, and the presence of bacilli was no reliable guide for prognosis. Animal tests were not made.

Suzuki and Takaki examined a great series of blood specimens and compared the results with the Pirquet test. They found acid-fast bacilli by the Stäubli-Schnitter method in 98 5/10 per cent. of five hundred and seventeen tuberculous cases of all stages, and also in 51 85/100 per cent. of fifty-four apparently healthy subjects. Reagents and all materials were sterilized. Both Ziehl and Much stains were used, but the latter method was discarded as unsafe. Animal tests were apparently made, but there is only a passing allusion to positive results, no details being given. The writers were impressed by the agreement between the microscopic test and the skin reaction. With all persons who gave a positive skin reaction there was also a positive blood test.

Liebermeister, in over one hundred tuberculous cases of different stages, obtained positive inoculation results in 48 per cent. By the Stäubli-Schnitter microscopic method, with Ziehl stain and aseptic precautions, he found acid-fast bacilli in each of fifteen cases of open tuberculosis and in eleven of thirteen cases of closed tuberculosis. In more than seventy cases, not clinically positive, several cases of scrofula, and also a long series of patients in various diseased conditions, not tuberculous, he obtained the same result. He concludes that these conditions have some connection with tuberculosis not understood, and suggests a "secondary" tuberculosis, analogous to the secondary stage in syphilis. Liebermeister notes that when several guinea pigs are inoculated at the same time with identical amounts of the same blood only one or two become tubercular. Therefore, one or two negative inoculation results do not exclude tuberculosis.

Rumpf, together with Zeissler, used the Schnitter method with oxalate in place of citrate, and a concentrated antiformin. All aseptic precautions were observed. He found acid-fast bacilli in 100 per cent. of twenty-five cases of tuberculosis, also in six former patients healed for twenty years, and in seven healthy persons, one of whom was scrofulous as a child. Of thirty-five guinea pigs inoculated intraperitoneally with microscopically positive blood only three became tubercular. In two of these the blood came from light closed cases, in the third from an open third stage case.

Hilgermann and Lossen, using the Stäubli-Schnitter method and all precautions with glassware and solutions, found acid-fast bacilli in seventeen (twenty-five per cent.) of sixty-four lung cases, all stages. No animal tests were made. Authors found no connection between positive blood and temperature conditions. The course of the diseases was less favorable in the majority of the cases with positive blood, but this could not be attributed to a beginning miliary process, since in many cases the positive blood test was followed by a temporary improvement.

Kennerknecht examined blood in 120 children, tuberculous, tuberculosis suspects and apparently healthy. By means of the Stäubli-Schnitter technique with the addition of absolute alcohol, new slides, all precautions with glassware and reagents, Ziehl and Gram stains, acid-fast bacilli were found in 109, or ninety-one per cent. of all specimens. There were very few bacilli, sometimes more in beginning or healing stages than at height of disease, and "granules" were included in positive findings. Of thirteen guinea pigs inoculated intraperitoneally all are said to have become tuberculous, but doubt is thrown upon the autopsy results by Bacmeister and also by Rabinowitz.

Klemperer, with Stäubli-Schnitter method and Ziehl stain, examined blood in thirty-nine cases of tuberculosis, suspected tuberculosis, other diseases and healthy persons. He found acid-fast bacilli in the majority of tuberculosis cases and tuberculosis suspects and in one case of liver cirrhosis, but never in healthy persons. Only characteristic well-stained bacilli were counted. These generally were few in number. Full details are given of aseptic precautions with solutions and glassware. No animal tests were made. Klemperer makes the observation that blood free from

bacilli dissolves in antiformin more slowly than blood in which bacilli are found. He argues from this a probable lessened resistance. In opposition to Suzuki and Takaki positive blood results were not found in all cases giving positive Pirquet reaction. Klemperer thinks the finding of bacilli of worth for diagnosis, but not for prognosis.

Ranstrom-Upsala used the method of Rosenberger and the Uhlenluth antiformin method, sterile glassware and all aseptic precautions. Of thirty-six cases of lung tuberculosis nine (twenty-five per cent.) showed acid-fast bacilli. This author alone seems to have found a definite ratio between temperature and positive blood test.

Bacmeister and Reuben with the Stäubli-Schnitter method, sterile glassware and filtered fluids, examined blood from "a great series" of cases of lung tuberculosis in the third stage, from fifteen cases in the first stage, from eight non-tuberculous subjects, and from a number of sound rabbits. In all of these specimens acid-fast bacilli were found. Granules were not counted. Blood from fifteen light and severe cases was inoculated into rabbits and in part into guinea pigs. These tests gave only negative results. The authors came to the conclusion that the chemical reaction of acetic acid and antiformin in the Stäubli-Schnitter method may give rise to appearances which simulate tubercle bacilli. The literature was reviewed and many theories discussed.

Lydia Rabinowitsch criticised the use by Bacmeister of rabbits instead of guinea pigs, as she found the latter much more suitable for the study of human tubercle bacilli. She also questioned the finding of bacilli in normal rabbits by Bacmeister and by Sturm. Rabinowitsch made the interesting observation that the blood of highly tuberculous animals frequently fails to produce tuberculosis when injected into guinea pigs, but that after the tuberculous animals have been injected with tuberculin the blood then gives a positive inoculation result. She quotes the experience of Suzuki and Takaki in this connection, and also observations of Virchow and Orth, published twenty-two years before, and agrees with the latter authors that the above result is probably due to the "mobilizing" of the bacilli through the action of tuberculin.

Bacmeister in a new series of tests comes to the same conclusion as Rabinowitsch on the above point. In fifteen cases of light tuberculosis he found acid-fast bacilli, but with all of these specimens animal tests were negative. Upon injecting the patients with tuberculin and taking blood at the height of the reaction he was able to produce typical tuberculosis in four (thirteen and three-tenths per cent.) of thirty inoculated animals. The acid-fast bacilli found in his earlier work with rabbits and guinea pigs, he now considered to have been "Fütterungs" bacilli.

Rosenberg examined blood in a series of surgical as well as pulmonary cases, also in healthy persons. In the healthy he found nothing in any case but in his other tests acid-fast bacilli were found in nearly every specimen. Very few organisms were present in any case and these were found after long search. There was no definite relation between the severity of the disease and bacilli in the blood, and Rosenberg considers

the presence of bacilli of no value for prognosis though specific as a means of diagnosis.

Nobécourt and Darré studied the question of bacillemia in tuberculous children of various ages with different forms of tuberculosis, using exclusively the method of guinea pig inoculation. Ten to twenty c. c. of blood was divided into equal parts and inoculated intraperitoneally into two or three guinea pigs.

Of forty patients, in acute, sub-acute and chronic stages, four only gave positive results. The first, a child of four years, showed a clinical picture of general tuberculosis. A guinea pig inoculated intraperitoneally died twelve days later and showed fresh granulations in the spleen containing acid-fast bacilli. A second animal inoculated with a fragment of this spleen developed general tuberculosis. Another specimen of blood taken eight days later, while the fever was still high, gave only negative results in two guinea pigs, one inoculated subcutaneously, the other intraperitoneally. This patient was later discharged, apparently completely cured and the diagnosis would have remained in doubt but for the positive animal test.

The second positive case was a little girl of four years with general tuberculosis of an extremely rapid type, the pulmonary symptoms predominating. Two guinea pigs inoculated intraperitoneally developed tuberculosis.

The third positive blood case was in a boy of nine years, with typical tuberculous meningitis. Blood taken three days after beginning of disease was negative in two pigs, but a second specimen, taken six days later, produced general tuberculosis by intraperitoneal inoculation.

The fourth positive blood was from an infant with acute tuberculous broncho-pneumonia. Two inoculated guinea pigs both developed general tuberculosis.

The authors point out the opposite inoculation results with different blood specimens from the same patient, and they emphasize the importance of the period of the disease when the specimen is taken, considering this a more essential point than the quantity of blood inoculated.

Querner used only animal tests. Injected guinea pigs intraperitoneally with sediment obtained by the Stäubli-Schnitter method from the blood of thirty-seven chronic pulmonary cases in all stages. In three cases two inoculations were made, making forty tests in all. The animals, killed twenty-seven to one hundred and forty-three days after inoculation, proved to be negative in every instance. The authors made control tests of the action of antiformin on tubercle bacilli, and found no lessening of virulence produced.

Krabbel made microscopic examinations of blood in thirty-five cases of tuberculosis of lung, third stage, tuberculosis of bones, of glands, or of skin. Using the Stäubli-Schnitter method, new slides, sterile glassware and stains constantly renewed, he found acid-fast bacilli in twenty (fifty-seven and one-tenth per cent.). No animal tests were made. Krabel finds no significance in positive blood tests, as they may be negative in advanced cases, and positive in cases getting well.

Brandes and Mau confined their investigations to surgical cases. With the Stäubli-Schnitter method and Ziehl stains they examined blood in forty cases of surgical tuberculosis of all stages (height and beginning of disease, and clinically healed). They used sterile glassware, new slides and water freshly distilled, and found acid-fast bacilli in eighteen (forty-five per cent.). Bacilli in blood were thought to indicate tuberculosis, but test was not considered of practical value.

De Verbizier examined blood in fifteen cases of tuberculosis by the Stäubli-Schnitter and Rosenberger methods, but found no acid-fast bacilli in any case. Fifteen guinea pigs inoculated intraperitoneally were all negative. The author concludes that bacillemia must be very rare in tuberculosis.

Rogers and Murphy, using the Stäubli-Schnitter and absolute alcohol method, tested blood in fifty cases of pulmonary tuberculosis, all stages, and in five apparently normal persons. They found acid-fast bacilli in all, morphologically identical with tubercle bacilli. Authors made numerous control tests. The water used was tested by adding albumen, centrifuging one-half to one honr, and examining the sediment, with results uniformly negative. They next added a small loopful of tuberculous culture to one c. c. of water, and a little albumen, and carried through same process as test specimens, after which the tubercle bacilli appeared on the slides in clumps. To eliminate crystals the authors made a series of tests with combinations of acetic acid, alcohol and albumen, acetic acid and solutions of alkalies and antiformin, but none of these tests gave crystals at all like the appearances found in the blood. These results are of interest as they are in opposition to the suggestion of Bacmeister and Reuben.

Dreesen tested blood in seventy cases of tuberculosis, tuberculosis suspects and apparently normal persons. Blood was drawn into freshly-distilled water, kept in the dark until next day and then centrifuged for one hour. One-half of sediment was treated with antiformin for two to three minutes, diluted with freshly-distilled water, centrifuged, and sediment examined with Ziehl stain. Only well-stained rods counted. Acid-fast rods and granules were found in seventy-four per cent. of the surely tubercular, fifty-five per cent. of the suspicious cases, and forty-six per cent. of the apparently normal cases. In marked contrast with these findings was the result of animal tests. The other half of the blood sediment above described was emulsified with two c. c. of sodium chloride and injected into two guinea pigs, the one pig being inoculated in the groin, the other in the peritoneum or liver. Of 128 pigs thus treated thirty-nine died prematurely, the other eighty-nine lived seven to eight weeks, and only one of all these animals gave any evidence of tuberculosis. This pig, inoculated from a case of advanced phthisis, showed true tuberculous lesions, and tubercle bacilli in smears. Tuberculin tests of all the other animals were negative.

Frankel made examinations in twenty-five cases, including tuberculosis second and third stages, catarrh, and other conditions. Eighteen specimens of blood were examined microscopically by the Stäubli-Schnitter method, with Ziehl, Much and Spengler strains. Four of these specimens

showed acid-fast bacilli. Forty-two guinea pigs were inoculated subcutaneously and intraperitoneally and of these only two developed tuberculosis. The blood injected in one pig was from a case of advanced phthisis, but in the other animal the blood was from a case of chronic bronchitis with no evidence of tuberculosis, and with blood and sputum negative microscopically.

Bernard, Debré and Baron, after trying various methods, selected the following for their work: Ten cubic centimeters of blood are taken from the vein into a sterile tube containing twenty cubic centimeters of thirty per cent. alcohol. Complete laking is obtained by the gradual addition of about thirty cubic centimeters of forty per cent. alcohol, with vigorous shaking. After centrifuging one-half hour the fluid is pipetted off, the sediment redissolved in forty cubic centimeters of forty per cent. alcohol, shaken energetically, and one to two drops of ten per cent. alcoholic solution of soda added. The fluid thus obtained is clear and but slightly viscous, if an excess of soda is avoided. The deposit obtained by centrifuging is then examined by the Ziehl stain.

The authors claim many advantages for this as compared with other methods. They examined forty-one specimens of blood from thirty-six tuberculous patients in all stages. Thirty-seven specimens were negative. In four cases the results were completely or partially positive. In the first case the blood was taken from a patient with tuberculous meningitis three days before death. Three apparently typical bacilli were found. Of two guinea pigs inoculated, one was negative three months later; the second developed tuberculous lesions containing acid-fast bacilli. Another pig inoculated with gland material from this animal died prematurely. The second positive blood specimen was from a case of miliary tuberculosis and showed a mass of apparently typical bacilli. Guinea pigs inoculated died accidentally. The third specimen was from a case of pulmonary tuberculosis with cavities, acid-fast bacilli in the blood. There was accidental death of the inoculated animal. In the fourth case the blood was from a patient with miliary tuberculosis, and the inoculated guinea pig developed typical lesions.

The authors question the accuracy of the methods usually employed, and especially the use of antiformin, as they have found acid-fast granules in the deposit at the bottom of a bottle of antiformin, and they suggest the possibility that acid-fast organisms may vegetate in this fluid.

Bernard, Debré and Baron made a second series of experiments using animals experimentally infected. Selected the guinea pig because it was possible to inject the entire blood of one animal into another. An inoculation dose was prepared by grinding up one centigram of a glycerine potato culture of tubercle bacilli in a sterile mortar, adding ten c. c. of sterile salt solution, and shaking into a homogeneous emulsion. Of this emulsion, one cubic centimeter, equaling one milligram of culture, was then injected into the external jugular vein of guinea pigs. Great care was used to avoid infecting the surrounding tissues, and the most careful technique was employed in the second part of the experiment when the blood was recovered from the carotid artery and introduced by transfusion

into other guinea pigs. Of twenty-three guinea pigs injected with the bacillary emulsion all sickened very quickly and severely, and showed the lesions of general miliary infection. The blood of these pigs, drawn at intervals of from twenty minutes to twenty-nine days, produced tuberculosis in every instance when introduced into other guinea pigs, and the microscopic blood examination was also positive in every case. Inoculations were also made of the urine and bile which had been collected with every aseptic precaution from the infected pigs. Leaving out the animals which died prematurely, all the urine inoculations were positive (pigs inoculated with urines obtained two, eight, ten, sixteen, twenty-one, twenty-two and twenty-nine days after injection), five out of eight inoculations of bile were positive (bile obtained two, sixteen, twenty, twenty-one and twenty-nine days after injection).

In fifteen guinea pigs injected with the same dose of tubercle bacilli subcutaneously the blood was found to be much less virulent, five out of sixteen guinea pigs being positive with blood drawn respectively at the seventh hour, the twelfth, eighteenth, twenty-second and thirty-first days. The authors conclude that after intravenous inoculation with tubercle bacilli the organisms remain constantly present in the blood until the death of the animal. The same is thought to be probably true of the urine, but not proven with regard to the bile. Bacillemia is found to be inconstant after subcutaneous injection.

Rist, Armand-Delille and Levy-Bruhl question the results obtained by Bernard, Debré and Baron. The former authors made tests in fifty cases, using the Stäubli-Schnitter method with Kurashiga modification. One cubic centimeter of blood was examined microscopically by Ziehl and Much stains, and nine cubic centimeters were injected intraperitoneally into guinea pigs. Blood from forty out of fifty cases gave a negative result by Ziehl and by Much stains, seven were positive sometimes by Ziehl and sometimes by Much. Guinea pigs inoculated with blood from these seven cases were all negative. Three specimens were positive both by Ziehl and by Much. In the first positive case the blood was from a patient with a cavity. A guinea pig killed in two months showed no sign of tuberculosis. In the second case, the guinea pig showed apparent lesions of peritoneal tuberculosis and granulation in liver and spleen, but a second guinea pig inoculated with this material remained healthy. In the third case, the guinea pig showed lesions of tuberculosis, and a few doubtful bacilli were found in smears. The authors point out the contrast between the work of Bernard, Debré and Baron, and their own results in which they have but one positive case among fifty tested. They are willing to admit a more or less rapid and transitory dissemination of the bacilli in certain cases, but consider this due to a condition of allergic sensibility. This condition, common to all the adult tuberculous, is incompatible with a septicaemia. The extraordinary percentage of bacilli in blood reported by some is thought by the authors to be due to technical errors.

During the last few months there have been three important contributions to this subject in articles by Elsaesser, Rothacker and Charon, and Lange and Lindemann.

Elsaesser, after an extensive review of the literature, gives the results of his own tests. He examined blood in forty-one cases of pulmonary tuberculosis, generally of a severe type, the blood being obtained not long before death. Many different methods and stains were employed, but acid-fast bacilli were found in only three cases, and in these the Zeissler modification of Schnitter's method was used together with Ziehl's stain. In twenty-five cases guinea pigs were inoculated with one c. c. of blood, two animals being used, one injected intraperitoneally, the other subcutaneously. Each pig was examined as it died. If both of a pair remained alive for some months one was killed and examined, the other left alive. In all cases these tests gave negative results.

Elsaesser calls attention to the strongly contrasting results of different workers, and also to the general discrepancy between the microscopic findings and the results of animal tests. He warns against conclusions based upon the microscope alone since the presence of tubercle bacilli in the blood is not proven without successful animal inoculation. At the same time, even with true tubercle bacilli, this proof may not always be obtainable, since, if the bacilli should be present in very small numbers the organism might be able successfully to cope with them.

Rothacker and Charon give a synopsis of the investigations of many authors as to the question of bacillaemia in the tuberculous, and report upon their own work in the examination of forty-six blood specimens from tuberculous patients in all stages. They used the Stäubli-Schnitter method and Ziehl stain, with every safeguard as to glassware, instruments, solutions, etc., and as an additional precaution the smears were not dried with filter paper. Many controls of all materials were made. In particular, control tests were made suggested by the work of Kahn with blood corpuscles, Kahn having stated that the stroma of red blood corpuscles, owing to their content of cholesterin and lecithin, were more or less acid-fast in nature, and thus might give rise to confusion with tubercle bacilli. The authors confirmed Kahn's statement as to the acid-fast property of lecithin and cholesterin, but found this to be weak in quality, and they did not agree with Kahn as to the possibility of confusion with tubercle bacilli. Of the forty-six blood specimens examined, all specimens from patients in the first stages were negative; about thirteen per cent. of those from the second stage, and sixty per cent. of those from the third stage were found to contain acid-fast bacilli. Forty-six guinea pigs were inoculated, but only one developed tuberculosis, this pig having been injected with blood from miliary tuberculosis. The authors conclude that the results of the Stäubli-Schnitter method are unreliable, and that animal inoculation is the only safe test for tubercle bacilli in the blood.

Lange and Lindemann, in a paper read before the recent meeting of the Freien Vereinigung fur Microbiologie in Berlin, gave the results of tests upon blood specimens from seventy-eight cases of pulmonary tuberculosis in all stages, two tuberculosis suspects, and two healthy persons. The authors were able to report only on the microscopic work, as the animal tests, begun some months before, were still in progress. Zeissler's modification of Schnitter's method was used, together with Ziehl's stain,

and full details are given of the most extreme care with glassware, solutions, instruments, etc. A thorough search was made for from one to one and one-half hours on each slide, in over one-third of the specimens, both investigators working together, and controlling each other, but all examinations were absolutely negative. Not only were there no acid-fast bacilli found, but no definite bacilli of any sort.

In contrast to Bacmeister's statement, the blood of four normal rabbits examined in the same manner, also gave entirely negative results.

As a control the authors made suspensions of tubercle bacilli in varying degrees of dilution in guinea pig blood, then centrifuged and found that in the dilution of one to one hundred millions the bacilli were still to be found microscopically. In the dilution of one to five hundred millions they were no longer detected. This "negative" sediment was injected into guinea pigs, still living at the time of report.

Of the 250 guinea pigs inoculated in the original blood tests thirty-eight had died, but only one of these had given any evidence of tuberculosis, and in this animal a careful search failed to show tubercle bacilli or any acid-fast appearances. The authors conclude that the many reports of positive microscopic results are to be received with much scepticism.

An interesting discussion, with opinions both in support and in opposition, followed the above paper.

Personal Experiments

Through the courtesy of Dr. Wilson, and the physicians stationed at North Brother's Island, and with Miss Smeaton's assistance, specimens of blood were obtained from patients in the second and third stages of tuberculosis, all giving a history of tubercle bacilli in the sputum.

A recent study of tuberculous sputa, which had been carried out in co-operation with Miss Smeaton by means of the Kinyoun Ligroin and Sodium Hypochlorite method, as compared with the Elliman-Erlandsen method, had shown the former to be an excellent method for the detection of tubercle bacilli in sputum. As a series of control tests had proved it to be of equal value for the finding of tubercle bacilli in blood specimens, this was the method chosen for the present work.

The technique pursued was as follows: With aseptic precautions from three to five cubic centimeters of blood were drawn from the vein at the elbow into a sterile tube containing two cubic centimeters of ten per cent. sodium citrate solution. To the blood and sodium citrate in the test tubes was added about three times the amount of sodium hypochlorite of such dilution as to contain fifty-six one-hundredths per cent. of available chlorine. One cubic centimeter of ligroin was next added, the mixture thoroughly shaken and allowed to stand over night at room temperature, when examination was made of the lower surface of the sharply-separated layer of ligroin. The centrifuge was not used, as originally recommended by Dr. Kinyoun, for in our work with sputum we had demonstrated, by numerous control tests, that if tubercle bacilli were present in the specimen they would be found without previous centrifuging in the soap-like stratum on the lower side of the ligroin layer, and in some cases our results had been found to be less favorable with the centri-

fuged portion of a specimen than with that portion which had simply stood over night after shaking.

New slides, which had been carefully prepared by boiling in soda solution, rinsing in water, rubbing with alcohol, and thoroughly firing in the flame, were covered with as much as possible of the material to be examined, the smears dried in the air, fixed by moderate heat and stained with the strong solution of carbol fuchsin recommended by Dr. Kinyoun:

Fuchsin, basic (Grubler).....	4 grams
Acid carbolic C. P. crystal.....	8 grams
Alcohol (95 per cent.).....	20 cubic centimeters
Water	100 cubic centimeters

Smears were decolorized with three per cent. hydrochloric acid in ninety-five per cent. alcohol, and methylene blue was used as counter stain.

All glassware was specially cleaned and sterilized and at first all solutions of every kind coming in contact with the specimens were passed through the Berkfeld filter. Later, when it became evident that the latter precaution was not necessary, unfiltered fluids were used as with the customary routine staining for tubercle bacilli.

Control tests were made during the entire course of the work by means of tubercle bacilli from pure cultures suspended in sterile salt solution. When dilute suspensions of these organisms were added in very small quantities to tubes of sterile blood, together with ligroin and hypochlorite, and the further process carried out exactly as with the test specimens, the bacilli were invariably found to be sharply localized beneath the ligroin layer, and could be readily demonstrated in smears even after several days in the hypochlorite mixture.

In all, blood specimens were taken from fifty tuberculous patients, two specimens obtained from one individual, one month apart, making a total of fifty-one specimens examined. Thirteen of these were from patients in the second stage, and thirty-eight from patients in the third stage of the disease, four of the latter being bed patients. Six patients have since died, the first three at intervals of from seventeen days to one month, the last three in from one month to five weeks after the taking of the blood specimens.

The most careful search was made for tubercle bacilli, from 150 to 300 microscopic fields being examined with each specimen but in all cases the results were absolutely negative, no acid-fast organisms of any kind being found in the smears. A few rods were occasionally seen, but never one which showed acid-fast staining, notwithstanding the precaution of having at least one smear from each specimen overstained and only so slightly decolorized that even partially acid-fast organisms must have been detected if present.

Owing to the invariably negative microscopic results no animals were inoculated, as it had been thought best to wait for some positive indications from the microscopic work before beginning animals tests.

As a preliminary to this work, examinations of blood taken from normal animals and human beings were made by the same method. In

this way specimens from thirteen horses, nine goats and three laboratory workers were examined with negative findings in all cases.

To summarize, the results of the present work are as follows: A total of fifty-one specimens from the blood were all negative. Of these, thirty-eight specimens were from the third stage, and thirteen from the second stage. All the specimens from normal controls, including thirteen horses, nine goats and three humans were negative.

Conclusions

The entirely negative results obtained during the present work by the microscopic examination of fifty-one specimens of blood, taken from patients in advanced stages of tuberculosis, are in agreement with the experience of a large number of observers. In other cases acid-fast bacilli have apparently been found in the blood of tuberculous patients by different investigators, but such bacilli have been reported present not only in tuberculous but also in normal individuals. Since organisms morphologically identical with tubercle bacilli are present occasionally in water and elsewhere, it is fair to assume that some, at least, of the acid-fast bacilli observed in blood were not tubercle bacilli, and where there were no confirmatory animal tests the proportion of specimens containing true tubercle bacilli must remain an open question.

Not only are animal tests indispensable in all cases before positive microscopic results can be accepted, but it is evident that in all microscopic work the utmost care must be exercised as to filtration of solutions and sterilizing of glassware, and also to make sure that no acid-fast organisms attach themselves to the slides during the various processes of fixing and staining.

According to the testimony of well-known observers, the results indicate that bacillaemia is occasionally present in tuberculosis in a wider range of cases than was formerly supposed. In view of the findings of Sturm, Rumpf, Liebermeister and others, it would seem that there must be some modification of the generally accepted opinion that tubercle bacilli are to be found in the blood stream only in cases of miliary tuberculosis.

On the other hand, results such as these stand in need of much confirmatory evidence, since in the great majority of cases no evidence of tuberculosis has been found, after the inoculation of animals with the blood of tuberculous patients.

In all, abstracts are here given of thirty-eight investigations, exclusive of the present experiments. In fifteen cases microscopic results only were reported, no animal tests being made. In seven series of tests the results of animal inoculation were entirely negative. In nine series the majority of the inoculation results were negative. In two reports all inoculation results are given as positive. In three others a large percentage of inoculation tests were positive, while in two papers positive inoculation results are reported without convincing details.

In view of the mass of contradictory evidence it is important that all possible proof should be given, including cultural tests with material from the infected animals in all cases of successful animal inoculation. At the

same time, the experience of Liebermeister and others must not be forgotten, as to the irregular action of the same blood upon different animals, since one or two negative inoculation results may not be sufficient proof that no bacilli are present in a given specimen.

Judging from the negative mircroscopic results in the present series of specimens from so many severe cases, it must be concluded that acid-fast bacilli are at least infrequent in the blood of tuberculous individuals, and while the nature of the organisms reported is a question of much interest, yet it is not a point which seems likely to prove of value in the diagnosis or prognosis of the disease.

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A COMPARISON OF RESULTS OBTAINED IN BINDING COMPLEMENT AT 37 DEGREES C. AND AT ICE-BOX TEMPERATURE IN COMPLEMENT-FIXATION TESTS

Archibald McNeil

In testing the sera of four hundred and sixty-six persons for syphilis using incubator temperature at 37 degrees C. for binding complement, 176 reacted positively, 273 negatively and 17 were doubtful.

The same sera when complement was bound at ice-box temperature gave 224 positive reactions, 212 negative reactions and 30 were doubtful, showing an increase of 10.3 per cent. of positive reactions.

Wassermann Tests

Complement Bound at 37° C.			Complement Bound at Ice-Box Temperature*		
	Number	Per cent.		Number	Per cent
Positive	176	37.7	Positive	224	48.
Negative	273	58.6	Negative	211	45.4
Doubtful	17	3.6	Doubtful	30	6.4
Total	466		Total	466	

* The temperature of the ice-box varied between 8° and 12° C.

On testing the same 266 sera for gonorrhea by the complement-fixation test, binding complement at 37 degrees C. in the incubator gave 134 positive reactions, 234 negative reactions and 98 gave doubtful reactions.

When complement was bound at ice-box temperature, 306 reacted positively, 101 negatively and 59 were doubtful, showing an increase of 36.9 per cent. of positive reactions.

Complement-Fixation Tests for Gonorrhea

Complement Bound at 37° C.			Complement Bound at Ice-Box Temperature		
	Number	Per cent.		Number	Per cent.
Positive	134	29.	Positive	306	65.6
Negative	234	50.	Negative	101	21.7
Doubtful	98	21.	Doubtful	59	12.6
Total	466		Total	466	

All of these 466 sera were taken from women who were inmates of a public institution. The majority of these women undoubtedly had been repeatedly exposed to venereal infection.

These tests were all made in duplicate to eliminate technical errors, and were all carefully controlled. The Wassermann technique with inactivated serum was used throughout.

In the syphilis tests the crude alcoholic extract antigen was used. In all tests where complement was bound at 37 degrees C., the sets were left for one hour in the incubator at 37 degrees C.

Where ice-box temperature was used to bind complement, a four-hour period was found to be the approximate optimum time for syphilis tests. Experience has shown, however, that when gonococcal or most other bacterial antigens are used, six hours in the ice-box is the approximate optimum time for binding complement.

The sera of several healthy horses injected with glanders vaccine gave strongly positive results with the complement-fixation tests when complement was bound at ice-box temperature; several weeks after completely negative results were obtained when a temperature of 37 degrees C. was used.

IS DIPHTHERIA FREQUENTLY A BACTEREMIA?

MATTHIAS NICOLL, JR., AND HARRIET L. WILCOX

In August last, Conradi and Bierast[1] of the Hygienic Institute of the University of Halle reported the results of an extensive examination of the urine of diphtheria patients. They call attention to the fact that, while diphtheria has been regarded as essentially a local disease, yet the Loeffler bacillus has frequently been isolated from the blood and organs after death, and occasionally from the blood during life, usually, however, in the agonal stage of the disease. On account of the technical and other difficulties of obtaining a sufficiently large quantity of blood from diphtheria patients, they sought to solve the question of diphtheria bacteremia by an indirect method, namely, an examination of the urine of those actively ill, and of convalescents. In all, 155 patients were examined, of whom fifty-four showed a positive urine. In six cases only, however, were virulence tests made, all of which proved positive. As the material was sent to the research laboratory at random, there was no possibility of following up a given case in order to study the question of the persistence of the bacilli by repeated examination. Of the 54 positive cases, 32 were female and 22 male; 36 children, 18 adults. Thirty-one urines were taken in the first week of illness, 10 in the second, 5 in the third, and 2 in the fourth. One was found positive in the ninth week of convalescence. The urine, 20 to 30 c.c. in amount, was taken by means of a sterile catheter with every precaution against contamination, and the centrifugalized sediment spread over one or two Loeffler plates and plates of Conradi and Troch. Both Loeffler and Neisser's stain were used for identification. The bacteria were found in normal as well as albuminous urine, showing that they pass through healthy as well as diseased kidneys. In most cases they were very few in number.

No clinical data are furnished in regard to the severity or outcome of the cases giving positive results. On the strength of their findings the authors make a plea for the disinfection of the urine of convalescents, until, by three negative cultures, it is shown to be free from diphtheria bacilli. They suggest that diphtheria of the skin and mucous membrane, which shows such a predilection for the genital and anal regions, is due to direct infection from the urine, and that the mystery that surrounds the origin of certain local epidemics of diphtheria may be explained in the same way.

Dr. R. Koch[2] of Frankfort, in December, reports the results of his examination of 111 urines from 26 patients. In 4 of these, which came from two patients, virulent diphtheria bacilli were found. In 10 urines from 5 other patients, diphtheria-like bacilli were found, which either could not be isolated in pure culture or else proved to be avirulent. In the original smears diphtheria bacilli were never recognized.

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[1] Conradi, H., and Bierast: Deutsch. med. Wchnschr., Aug. 22, 1912, No. 34.
[2] Koch, R.: Deutsch. med. Wchnschr., Dec. 12, 1912, No. 50.

The urine was passed into a sterile vessel after thorough cleansing of the meatus with mercuric chlorid solution. In 74 examinations made on 19 patients suffering from mild or moderately severe diphtheria—in one case 14 examinations were made in 25 days—no suspicious organisms were found. In 5 other patients diphtheria like organisms were found which proved to be avirulent; 2 of these were fatal cases. Post-mortem examination of the urine proved negative.

Of the two positive cases, both ended fatally from the toxemia of the disease and cardiac paralysis. The first showed negative urine on the first two examinations, virulent diphtheria bacilli being found on the seventh day, the patient dying on the following day. In the second case they were found on the fifth day of the disease and the following day, being absent on the next five days and present on the twelfth day again, the patient dying the next day. The appearance of the bacilli in the urine was in neither case coincident with change in the general symptoms. The presence or absence of albumin in the urine had no bearing on the presence of bacilli.

Nineteen cases of scarlet fever complicated by diphtheria were examined as controls. In four of them the urine contained diphtheria-like bacilli which proved to be avirulent. They believe that only animal tests can be relied on for the identification of diphtheria-like bacilli in the urine. While Loeffler bacilli may occasionally occur in very severe cases, the fact is not of practical interest, as such patients, being confined strictly to bed, would not be likely to be a source of danger through infected urine.

The latest writer on the subject is Walter Beyer[3] of Rostock, whose reported results are little short of startling. He examined nineteen cases of diphtheria daily, or every second day, from the active stage of the disease to late convalescence. Forty c.c. or more urine were taken with proper precaution against contamination, and the centrifugalized sediment poured over a Loeffler plate. Practically every urine examined showed diphtheria bacilli at each examination; their number being greatest during the active stage of the disease and gradually diminishing. In the case of eight convalescents, four of whom had recovered from their illness, three and one-quarter to three and one-half months previously, and the others eight, six, five and four weeks, respectively, the urine showed the continued presence of diphtheria bacilli, six strains of which were said to have been virulent for guinea-pigs, the other two avirulent. In all of the eight cases cultures from the tonsillar crypts showed dyphtheria bacilli. It is to be noted that there is nothing said by Beyer or Conradi as to their methods of testing for virulence; that is, as to whether anitoxin was used in the test.

Beyer believes that it has been established beyond doubt that in the early stages of diphtheria, at least, the disease is essentially a bacteremia. Whether the organisms found in convalescents constitute also an evidence of a continued blood-infection he does not undertake to say.

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[3]Beyer, Walter: München. med. Wchnschr., Feb. 4, 1913, No. 5.

Our series of cases comprises 54 patients with 56 examinations of urine. These were divided into three groups roughly corresponding to the type of disease which characterize the clinical material from which the three observers quoted obtained positive results. Thus the first group, 25 in number, consisted of routine cases; the second, 21 in number, cases of the severer type with a great deal of membrane and showing symptoms of toxemia; third, 8 in number, cases of recent convalescents. All were patients at the Willard Parker Hospital.

In the first group, there were 5 adults and 21 children, the ages of the latter ranging from 2 to 13 years. There were 18 males and 7 females. Twelve patients were in the first week of the disease, 7 in the second, 3 in the third, 1 in the sixth week and 2 not determined. Only 1 or 2 of these cases were of any severity. The urine proved negative in every case.

In the second group of 21 cases there were 4 adults and 17 children, the latter 2 to 11 years, 16 males and 5 females. Twenty were ill for a week or less at the time of taking the urine and 1 with croup and cervical adenitis for sixteen days. Two of this group proved positive. One, Edward M., 8 years of age, two days ill, had thick membrane covering the tonsils, fauces, uvula and posterior walls of the pharynx; there was also nasal discharge and marked toxemia. The serum tube and ascitic broth showed Loeffler bacilli which proved to be virulent. A specimen taken one week later was negative. The patient made an uneventful recovery. A second patient, Sam G., aged 5 years, had been ill three days. The patient had thick membrane on both tonsils, uvula and pharynx with profuse purulent nasal discharge. The cervical nodes were swollen and the patient very toxic. The serum tube and broth showed virulent Loeffler bacilli. The second specimen, taken eight days later, proved negative. Neither of these patients were catherized on either occasion. Of the remainder of this group, from one, Hoffman's bacillus was isolated, and from a case of diphtheria complicating scarlet fever and sepsis a specimen taken by catheter showed diphtheria-like bacilli, which, however, proved not to be diphtheria. All the other cases were negative.

In the first two groups the female patients were catheterized, 20 or more c. c. of urine being drawn into a sterile centrifuge tube. In the case of males the first part of the urine was discarded and the residue passed into sterile centrifuge tubes through a sterile glass funnel. In one or two instances very young males were catheterized. In three cases only 8 to 12 c. c. of urine was obtained. The centrifugalized sediment was treated as follows:

One c. c. was drawn off by pipet, 0.8 of which was put into 3 to 4 c. c. of ascitic broth and the remaining 0.2 poured over a Loeffler serum tube and incubated. Colonies developing on the tube were examined in the routine way, and if any pellicle developed on the broth, it was stained with Loeffler's methylene blue and examined.

In the third group, although we had every confidence in our technic in producing equally if not more definite results than theirs, it was thought advisable to follow exactly that of the three writers quoted. The patients were catheterized after thorough cleansing of the meatus with

mercuric chlorid solution and 35 to 40 c. c. of urine drawn into a sterile container. The centrifugalized sediment with 1 c. c. of the urine was drawn off by pipet and one-half of the quantity allowed to flow over each of two Loeffler plates previously tested with a known strain of Loeffler bacillus. In addition, in four cases 5 c. c. of the remaining urine was put into 75 c. c. of ascitic broth.

In this group there were eight patients, five of whom were females, five children and three adults. With one exception, they were all cases of clinical diphtheria of a pronounced type; the exception was a case which showed only a markedly congested pharynx from which cultures taken on several different days showed typical diphtheria bacilli. The patients had been ill from three to twelve days and were mostly afebrile at the time of the test. All showed the presence of diphtheria bacilli on culture in the throat.

In three cases there was no growth on the plates after twenty-four hours; in the other five, only colonies of cocci were present, probably staphylococci. The ascitic broth also proved negative.

CONCLUSIONS

The two positive findings in our series may well have been due to accidental contamination at the time of taking the urine, especially as a catheter was not made use of in either case. We conclude, therefore, that diphtheria bacilli may occasionally gain access to the blood and be excreted in the urine in very severe cases of diphtheria with marked ulceration of the mucous membrane of the pharynx and tonsils. This fact is of theoretic interest, but of little practical importance to the physician. Finally, we believe that identification of diphtheria bacilli in the urine should not rest on morphologic characteristics alone, but be confirmed by isolation and animal inoculation controlled by the use of diphtheria antitoxin.

PRESENT-DAY OPINIONS OF THE VALUE OF THE SO-CALLED INCLUSION BODIES IN SCARLET FEVER

Matthias Nicoll, Jr.

The inclusion bodies described by Professor Döhle, of Kiel, as found in the polymorphonuclear leukocytes of scarlet fever blood, have since become the subject of extensive study in Europe and, to a less extent, in this country. Döhle was of the opinion that these bodies would be found pathognomonic for scarlet fever. Kretschmer, of Berlin, shortly afterward confirmed these findings. Dr. Anna Williams and I also undertook their study at the Research Laboratory of the Department of Health, and came to the conclusion that while Döhle's claim of specificity for them was not justified, since identical findings occurred in a number of other conditions, nevertheless the determination of their presence or absence was of decided value to the clinician in helping him to make a differential diagnosis between scarlet fever and conditions which frequently simulated it. This position we still maintain in spite of the adverse opinion of many observers and of the fact that a more extensive study of very mild cases of scarlet fever and of control cases of other conditions has led to a somewhat lessened regard for their value.

The literature of the subject has now become so extensive that only a cursory review of it is here possible. In general, it may be stated that, with the exception of Kretschmer, Professor Kolmer, of Philadelphia, and ourselves, all the writers have little to say in favor of Döhle's work. Thus Bongartz, of Nuremberg, in a study of 80 cases, including 21 normal bloods, and the remainder of scarlet fever, diphtheria, measles, pertussis and even diarrhea, finds inclusion bodies in 81 per cent. of normal blood, and in 91½ per cent. of the others, and states that in the blood of children inclusion bodies are found in the majority of cases, and that they are greatly increased in febrile conditions and therefore cannot be pathognomonic for scarlet fever. Glomset, in the *Journal of Infectious Diseases* of November, 1912, goes still further, stating that by shaking up normal blood the bodies may be made to increase in number. Contrary to all other observers, he makes no distinction between the so-called inclusions and detached or pediculated portions of the nucleus, notwithstanding the difference in the action of the two toward various stains. He believes that the bodies reach their maximum on the fifth day, which he regards as the height of the disease. The article contains the photograph of measles blood, said to have been from a hemorrhagic case which differs in no respect from the blood seen in an early case of pronounced scarlet fever.

Bélak, of Budapest, believes that only when a suspected case of scarlet fever shows no inclusions in the blood can this disease be excluded, the presence of inclusions in any case having no value for diagnostic purposes. The most recent article on the subject is that of Grainger and Pole, of London, who examined a large number of scarlet fever cases and controls. They find that a carbol methyl blue stain gives

better results than the generally used Manson and state that Döhle's inclusion bodies will probably be found in every true case of scarlet fever during the first four days, and that the absence of these bodies practically excludes this disease. They find them, however, in a large proportion of cases of diphtheria, measles and tonsillitis, and therefore believe that they are of no value in making a differential diagnosis between these diseases and scarlet fever. It is stated that a number of measles cases were complicated by broncho-pneumonia, but that some uncomplicated cases also showed inclusions. They were not found in toxic rashes, and no cases of German measles were examined. They conclude that it is impossible to diagnose scarlet fever by blood examination alone.

In contrast to these opinions Kolmer, of Philadelphia, is in essential agreement with Kretschmer and ourselves. He first pointed out, however, that in the first four or five days of diphtheria, typical inclusions could be found in quite a large percentage of the cases, and after that but seldom. This fact, which escaped us in our first studies, I have since been able to confirm. He finds inclusions in the majority, but not all cases of scarlet fever in the first few days and regards them of value in diagnosis. Kretschmer, in a second communication in November, reiterates his belief in the value of the bodies, and states that with the exception of septic conditions, those in which they are found other than in scarlet fever are not likely to be confounded clinically with the latter.

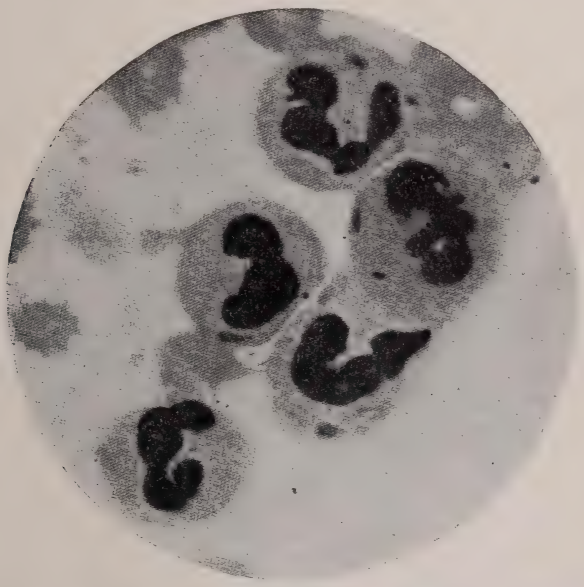
This diversity of opinion must be accounted for on other grounds than incompetence or prejudice on the part of the different observers, and I believe is due to the following causes:

(1) That the majority of workers have chosen to regard a given case as positive if, after prolonged search, one or two polymorphonuclears show a single body of whatever size or shape, thus using the term in an absolute, rather than in a relative or quantitative, sense. Now it is perfectly true that by examining a great many cells, even in normal blood, an occasional inclusion may be met with. Thus, in a series of twenty bloods from as many supposedly healthy children, Dr. Williams and I, by examining a hundred or more leukocytes in each case, found in two cells one in each of 2 cases, an inclusion, one very small and the other a fair-sized mass.

At the Research Laboratory from the beginning, as a routine, even if arbitrary, method, we have been in the habit of expressing an opinion of a given blood by using a series of plus marks from four to one, according to the number of cells showing inclusions and the total number of inclusions, and the plus minus sign + — to designate a case in which a few are found, but not sufficient to justify a diagnosis.

(2) Many observers have drawn their conclusions solely on the findings in blood sent to the laboratory, sometimes from another city, and without clinical data of the illness from which the patient suffered. From our own experience, I believe that in a study of this kind no really definite conclusions can be reached by such a method. Within a few steps of the Research Laboratory we have ready access to a vast amount

of clinical material among the infectious diseases, and the laboratory work has been strictly controlled by the personal study of the patients in nearly all cases which showed unusual blood characteristics, and these have been readily explained by this proceeding. As an example, an infant of nine months showed no inclusions in the blood, and ten days after admission to the scarlet fever service developed typical scarlet fever. Again, a blood smear from a measles case was sent for examination and found to be unquestionably positive. On visiting the patient, I found that it had a marked nasopharyngeal diphtheria, clinically and by culture. These two cases from the blood examination alone, and without clinical control, would have sufficed to upset our regard for the value of the bodies. Such instances have been very numerous.



Blood from two cases of scarlet fever taken the fourth day. The inclusions are seen in the leukocytes less deeply stained than the nucleus. (Manson's stain.)

We have continued to study the inclusion bodies during the past year and have now examined 300 to 400 cases of scarlet fever, usually in the active stage of the disease, that is, during the first week. The majority of the smears have come from the scarlet fever service, but quite a large number from private sources for diagnosis, the history of the case being later given to us by the physician.

Up to a short time ago, I was convinced that every case of true scarlet fever up to, and including, the fourth day would show in the blood a sufficient number of inclusion bodies of all types to justify a differential diagnosis between scarlet fever and such condition as, in my opinion, show no such findings.

During the past winter there have been an unusual number of very mild cases of the disease with very little involvement of the throat, low fever and atypical tongues. In a recent study of 33 such cases, a number of which were examined daily from the first to the fifth day, five showed too few inclusions to justify calling the blood smear positive. Two of these cases were, to my mind, doubtful, and were possible examples of German measles; the others were, undoubtedly, mild scarlet fever. In some instances the inclusions were more pronounced on the third or fourth day than the first.

In a small percentage of true scarlet fever, then, practically negative results may be expected.

Pathologic conditions, which, even in the hands of expert diagnosticians, may justifiably be confused with scarlet fever, are, first of all, sepsis, whether due to surgical or accidental traumatism, or complicating a disease, showing a scarlatiniform eruption, and just as there is absolutely no way at present to differentiate scarlet fever from sepsis by clinical symptoms, so, unfortunately, are the blood pictures identical.

Serum rashes of whatever variety show no inclusions in the blood, and I wish to emphasize at this time that by this statement now and hereafter I mean no inclusions which would serve to confuse this disease with scarlet fever. If such are present in a case of diphtheria, to which antitoxin has been given, and during the first five days of the disease, they may be due either to diphtheria or the streptococcus infection associated with it in the throat; therefore positive findings at this period are of no value. Negative findings practically exclude the presence of scarlet fever. After this period, a positive finding should make us very suspicious of its existence.

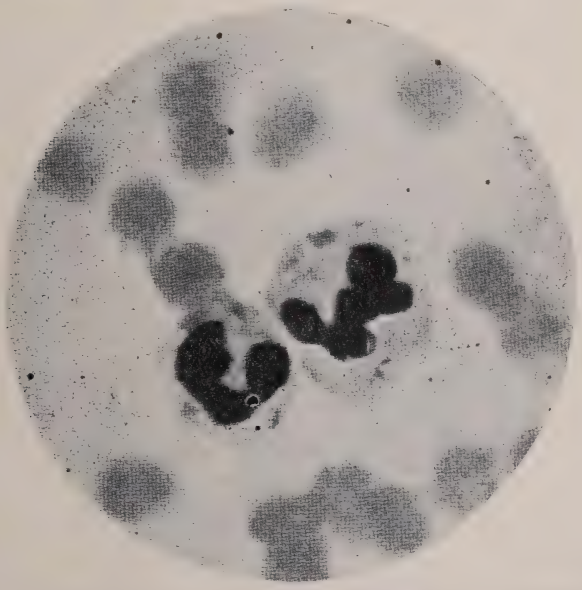
Toxic rashes due to other causes than serum show no inclusions.

German measles, so frequently confused with scarlet fever, shows no inclusions. This statement is based on an examination of 40 or 50 cases, in several of which there was present a decidedly red throat.

Measles blood is peculiar. In an examination of some 40 cases of uncomplicated measles from the preëruptive stage through the first week, not one has shown a blood picture similar to that of scarlet fever, although it is true that there are regularly present in the polymorphonuclears tiny granules which are quite unlike the relatively large masses found in scarlet fever. For which reason I have no hesitation in stating that the picture of measles blood which accompanies the article of Glomset, already referred to, is misleading as typical of uncomplicated measles, although I have not had an opportunity of examining a case of the hemorrhagic variety, which this was said to have been.

Acute Follicular Tonsilitis.—The necessity for a differential diagnosis between this condition and scarlet fever may occasionally arise, especially when the tonsilitis is accompanied, as it sometimes is, by an erythematous eruption. From the fact that the streptococcus is not infrequently associated with the staphylococcus and other organisms in this condition, one might expect that the inclusion bodies would occasionally be found. The blood examination in tonsilitis have not been sufficiently numerous

to justify a positive conclusion in regard to the matter, yet in the 15 or 20 cases studied no typical inclusions have been found. Another condition of less frequent occurrence and which may best be described as a streptococcus angina, and which clinically resembles very closely, if not exactly, the angina of scarlet fever, cannot be distinguished from the latter by blood examination, as typical inclusion bodies are regularly found. I have seen a number of such cases among the medical staff at the scarlet fever hospital and the nurses, and may state that it is often impossible to classify them clinically. The cases are usually suspected of being diphtheria, a culture is taken and antitoxin given. The cultures show a great predominance of streptococci, then scarlet fever is anticipated and a rash is diligently searched for without result. My personal feeling in regard to such cases is that unless scarlet fever can be absolutely excluded it is safer to regard them as atypical examples of the disease and protect others from exposure until at least a week after the throat has become normal.



Blood from two cases of scarlet fever taken the fourth day. The inclusions are taken in the leukocytes less deeply stained than the nucleus. (Manson's stain.)

That these bodies may be found in certain pneumonias, erysipelas, pulmonary tuberculosis of an active type, typhus fever, and other conditions, is not pertinent to the inquiry regarding their value as a means of differential diagnosis between scarlet fever and a disease resembling it, provided that neither is complicated by one of the diseases mentioned.

This means of differential diagnosis has been put to a practical and very exhaustive test, not only in the diphtheria and scarlet fever hospital services of the city, but in a good many cases in which blood smears

have been sent in from private sources, and has given decided satisfaction. As an example of a case in which it would have proved a great service if carried out, I may cite the following: A child became ill with diphtheria, was given anti-toxin and isolated from the rest of the family. Ten or twelve days later a scarlatiniform rash appeared on the body with very few constitutional symptoms. Cultures had shown the child to be free from diphtheria, the rash was regarded as due to antitoxin, and she was allowed to return to her brothers and sisters. No blood examination was made. Four days later two of these contracted scarlet fever, and one of them died. The first child also died of scarlatinal nephritis. Here then, is an instance where two lives might have been saved by a simple procedure not taking more than five minutes to perform.

To sum up, the findings of typical inclusion bodies in a case resembling scarlet fever before the fourth day of the disease may mean scarlet fever, sepsis or severe streptococcal angina. Negative finding practically excludes the existence of scarlet fever. In the course of diphtheria, for which antitoxin has been given, positive findings before the seventh day are not diagnostic of scarlet fever. After this time a positive finding in the case of a scarlatiniform rash should make one suspect very strongly the existence of a complicating scarlet fever.

There is at present no short-cut to the diagnosis of scarlet fever. All the clinical symptoms and signs must be taken into consideration in reaching a conclusion, and among them the determination of the presence or absence of inclusion bodies in the blood has a not unimportant place.

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THE VALUE OF ABSORPTION METHODS IN THE WASSERMANN TEST

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The presence of antisheep amboceptor in human serum has been considered by various serologists a source of error in the Wassermann test. The amount of serum used in the test may contain from zero to twenty units and the excess of amboceptor thus introduced may result in such an increase in hemolysis that a positive reaction is lost. According to Bailey[1], from five to fifteen extra units may cause a false negative result. Instead of further increasing the excess by the addition of the regulation two units of amboceptor in the course of the test, the natural amboceptor content of the serum may be depended upon for hemolytic action, as in the Bauer modification.[2,4] Since, however, the amount of natural amboceptor is a variable quantity there is no certainty of the hemolytic system being in balance and the reaction may still be inaccurate. A more satisfactory solution of the problem is the absorption of the natural amboceptor from the serum, so that the balance of the hemolytic system may be assured by the addition of a known quantity of amboceptor. Methods of absorption have been described by Rossi[9], Jacobaeus[5], Mintz[6] and others.

Other serum components as well as natural amboceptor may interfere with a positive reaction. Wechselmann[11] has called attention to the presence in heated serum of complementoids, which, though they have no hemolytic action, retain haptophore groups, hence are capable of being bound by the antigen-antibody combination and prevent the latter from binding complement, which is thus left free to hemolyze the cells subsequently added[7]. Noguchi[8] and others have expressed doubt as to the complementoid nature of the serum components thus preventing a positive reaction but agree that the accuracy of the test is increased by their absorption, which may be accomplished by treating the serum with barium sulphate. According to Jacobaeus[5] complementoids as well as natural amboceptor may be absorbed by digestion with sheep cells.

In order to determine the value of absorption methods in routine Wassermann work two series of tests were carried out at this laboratory, which though too brief to be conclusive are suggestive.

Series I

The technique employed in this series was the classical Wassermann[8], with the exception of the size, the quantities being proportionately the same, but the final volume 0.5 c. c. instead of 5 c. c. The first incubation was in the water bath at 37° C. for half an hour, the second, after the addition of 0.1 c. c. of 5 per cent. sheep cell suspension and two units of amboceptor, in the water bath for one hour. The tests were then allowed to stand in the ice box over night before being read. A test was considered positive if 0.02 c. c. of serum fixed complement completely though 0.01 c. c. might show no fixation.

One hundred sixty-two specimens of serum were tested for natural antisheep amboceptor as follows: 0.02 c. c. of inactive serum, 0.1 c. c. of 10 per cent. complement, 0.1 c. c. of 5 per cent. sheep cell suspension and 0.28 c. c. of physiological salt solution were mixed and incubated in the water bath at 37° C. for one hour. Hemolysis, partial or complete, indicated the presence of natural antisheep amboceptor in seventy-seven specimens, 47.5 per cent. The sera that were tested for natural amboceptor after absorption had no hemolytic effect on sheep cells. The natural amboceptor was absorbed according to Rossi's method[9], i. e., three parts of inactive serum and one part of closely packed, washed sheep cells were mixed, kept on ice for from twenty to thirty minutes and then quickly centrifuged. The supernatant serum was tested as usual. Of forty-seven specimens that gave a negative Wassermann reaction before absorption five, 10.6 per cent., were positive after absorption. Two were from undoubtedly specific, active cases, one was from a case of latent syphilis and two were from cases that gave a suspicious history. Five cases with no luetic history remained negative after absorption.

Wechselmann[11] has recommended treating all sera to be tested for syphilis with barium sulphate, to absorb the complementoids and thus increase the binding of complement. The technique of the absorption is as follows: Nine parts of inactive serum, thirty parts of physiological salt solution and five parts of a 7 per cent. suspension of freshly precipitated barium sulphate are shaken together, incubated for one hour at 37° C. and centrifuged. Of the supernatant fluid, ten times the regular amount for the Wassermann test must be used. Wechselmann[11], Wolff[12] and others have emphasized the non-suitability of commercial barium sulphate for absorption purposes; but they have not stated how fresh a precipitate should be used. We have found a precipitate four weeks old as effective as a perfectly fresh one. Since, however, a suspension of the barium sulphate is apt to become anticomplementary in three or four weeks, it is advisable to make up a fresh suspension at least once in two weeks. A simple method of precipitating barium sulphate is described by Wolff[12]. Mix 15 c. c. of sodium sulphate and 10 c. c. of barium chloride. Centrifuge after precipitation has taken place, draw off the supernatant fluid and wash the residue, barium sulphate, once in physiological salt solution. After centrifugalization suspend the barium sulphate sediment in 25 c. c. of physiological salt solution, which makes the proper dilution for use in absorption.

We treated with barium sulphate forty-three specimens that had given a negative reaction in the unmodified Wassermann test, six of which—a percentage of 13.9—gave a positive reaction after absorption. Five specimens from which a history of syphilis could be absolutely excluded were even more clearly negative after absorption than before. Seven positive sera that were treated with barium sulphate lost no binding power thereby. That barium sulphate itself does not bind complement was controlled in each set of tests by a treated negative serum or by barium sulphate and salt solution alone. Eight specimens with a suspicious gonorrheal history that had given a negative reaction in the unmodified

complement fixation test for gonococcal antibodies were treated with barium sulphate, after which two of them reacted positively, suggesting that Wechselmann's absorption method might be of as much value in the serum diagnosis of gonorrhea as of syphilis.

At the suggestion of Dr. A. McNeil, a combination of the Rossi and Wechselmann methods was tried, to determine the effect of absorbing both natural amboceptor and the other serum components that prevent a positive reaction. Sixty-six specimens that had given a negative Wassermann reaction in the unmodified test were treated first with sheep cells and then with barium sulphate; eighteen of them, 27.2 per cent., were positive after absorption. Exactly the same percentage was obtained in similar tests for gonococcal antibodies, three out of eleven negative specimens being made positive by absorption with sheep cells and barium sulphate.

Wolff[12] has emphasized the value of the barium sulphate treatment for the absorption of complement as well as complementoid. He also advises the use of active rather than inactive serum, as heating at 56° C. destroys some antibodies, thus weakening a positive reaction. We treated with barium sulphate twenty-eight specimens of active serum that, inactivated, gave a negative Wassermann reaction. Nine of them, 32.1 per cent., gave a positive reaction after absorption. Although this percentage is higher than in any of the series of tests with inactive serum, the use of active serum does not seem to be advisable. Three cases from which a history of syphilis could be absolutely excluded gave a positive reaction, and in at least one other instance it seemed highly probable that the binding was non-specific.* In eleven instances 0.1 c. c. of serum bound *more complement than* 0.2 *c. c.* Though this phenomenon was not constant, it occurred so frequently as to render the test unreliable.

Thus, it appears that by the application of absorption methods in the Wassermann test the number of positive reactions is increased. Wolff's method of treating active serum with barium sulphate gives the highest percentage, but is not, according to our experience, reliable. The combination of Rossi's and Wechselmann's methods gives a fairly high percentage of positives; and as far as may be judged from the results of our few tests there is no danger of thereby rendering the Wassermann reaction oversensitive. The effect of barium sulphate on sera free from syphilitic and gonococcal antibodies should, however, be tested at length before the use of barium sulphate in the routine sero-diagnosis of these venereal diseases is recommended. Rossi's method of absorbing natural amboceptor from the serum to be tested is perfectly safe, and its practical value is indicated by this series of tests.

Series 2

After the adoption of a change in the technique of the Wassermann test that resulted in an increase in the number of positive reactions, a second series of tests was made to indicate whether or not the natural anti-sheep amboceptor in human serum might still be a source of error in the

* Jacobaeus describes a similar experience.

diagnosis of syphilis. The readings were made as soon as hemolysis was complete in the antigen control (double the amount of antigen used with the serum) and in the serum control (0.04 c. c., double the maximum amount of serum used with the antigen), instead of after an hour's incubation in the water bath and a night in the ice box, as heretofore. The system of reading was the same as in the previous series of tests.

Of two hundred twenty-nine sera tested for natural antisheep amboceptor, sixty-four, 27.9 per cent., were found to contain one or more units.† Fifty-two of these sixty-four were treated with packed sheep cells, according to Rossi's method,[9] for the absorption of the amboceptor. In one case, serum that fixed complement only partially before absorption gave a ++ reaction‡ after absorption. Of the thirty-seven cases that were negative before absorption, thirty-four remained negative and three gave a doubtful reaction after absorption. No negative became positive.

Thus, by the absorption of natural antisheep amboceptor from the serum of forty-three doubtful or negative cases, the diagnosis was changed in four instances, becoming positive in only one. Twenty-six of these same specimens, untreated, gave positive reactions by ice box fixation.§

Conclusions

Natural antisheep amboceptor is frequently present in human serum. An excess of amboceptor in the Wassermann test may result in false negative reactions. By the absorption of the natural amboceptor the binding power of some sera is increased, especially if the amboceptor has a long period of time in which to act, as is the case when the second incubation of the test is for one hour and the readings are not made until the following day. If, however, the readings are made as soon as antigen and serum controls are completely hemolyzed, an excess of amboceptor makes little difference in the results. Thus, the method of reading tests seems to determine the importance of absorbing natural amboceptor.

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† By a unit of amboceptor is meant the smallest amount that with 0.1 c. c. of 10 per cent. complement completely hemolyzes 0.1 c.c. of 5 per cent. sheep cell suspension when incubated for one hour in the water bath at 37° C.

‡ 0.02 c. c. of serum gave complete fixation, 0.01 c. c. complete hemolysis.

§ After the mixture of serum, antigen, complement and saline the tests were set in the ice-box for four hours, then filled with sensitized cells and incubated at 37° C. until readings could be made.

A CONSIDERATION OF THE PANCREAS AND ITS DUCTS IN CONGENITAL OBLITERATION OF THE BILE-DUCTS

ALFRED F. HESS

Congenital obliteration of the bile ducts is a rare disease, but hardly sufficiently uncommon to warrant the report of a single case, unless it presented unusual symptoms, or served as the ground-work for some new conception of the symptomatology, pathogenesis, or pathology of this obscure condition. The excuse offered for this presentation of a case is that the application of a new method of study during life led to a somewhat broader point of view of this pathological condition. In the course of a clinical study, carried on at intervals for about six weeks on an infant afflicted with this condition, the dudoenal catheter was frequently employed, in order to obtain information as to the excretion into the intestine of bile as well as of the pancreatic ferments. This catheter has been described in connection with a study of spasm and stenosis of the pylorus,[1] of icterus neonatorum,[2] and of the flora of the duodenum in infants,[3] so that it is not necessary for the technic to be again reviewed in detail. The accompanying cut (Fig. 1) shows the simple apparatus, which consists of an ordinary Nélaton No. 15 (F) catheter, marked in an appropriate manner, and of a glass aspirating bulb. As has been stated in previous articles, this catheter can be readily and rapidly passed into the stomach, and through the pyloric sphincter into the duodenum, in infants, from the day of birth to the age of about 2 years. By this means bile and the ferments of the duodenum and pancreas may be obtained for examination.

REPORT OF CASE

M. E. was admitted to the hospital Dec. 19, 1911, when 7 weeks old.

Family History.—First child, pregnancy and delivery normal; no history of miscarriages.

Present History.—History of early and increasing jaundice; exact date of onset uncertain; nursed by mother irregularly.

Examination.—At date of admission the infant was markedly yellow, fairly well nourished, weight 9 pounds 4 ounces. Liver at level of umbilicus. Spleen one finger above crest of ilium, edge sharp and soft. Lungs, heart, and general abdominal examination negative. Urine negative, except for the large amount of bile pigment. Stools clay colored. Blood examination showed 6,000 leukocytes, 65 per cent. mononuclear cells, 35 per cent. polynuclears.

December 20. Child being nursed every four hours. To-day traces of blood following rectal irrigation.

Duodenal catheter passed 1½ hours after nursing, and 2 ounces of uncoagulated milk evacuated. Duodenum easily entered and 3 c.c. of fluid obtained in the course of one hour, slightly acid, and faintly bile-tinged. Stool clay colored with traces of bile.

[1]Hess, A. F.: Am. Jour. Dis. Child., 1912, III, 133.
[2]Hess, A. F.: Am. Jour. Dis. Child., 1912, III, 304.
[3]Hess, A. F.: Jour. Infect. Dis., 1912, X, No. 5.

December 21, Catheter passed a few minutes after nursing. Stomach contents acid, Congo ++. Duodenal contents contain slight amount of bile and food strings (Cannon).

December 22. Jaundice more intense. Catheter passed five hours after nursing. Stomach empty. Duodenum empty after twenty-five minute test.

December 23. Catheter passed three and one-half hours after nursing. Stomach empty. Duodenum empty after a one-half hour test.

December 24. Condition the same. Catheter passed an hour after nursing. Acid, Congo + milk evacuated. Mucoid fluid containing some bile found in duodenum.

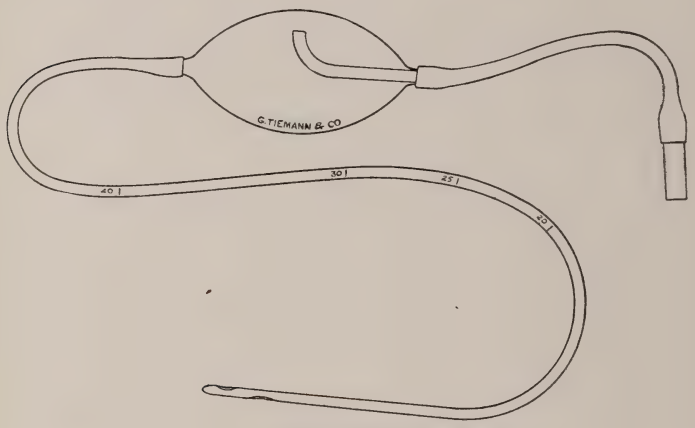


FIG. 1.—Infant duodenal catheter.

December 25. Jaundice increased. Infant sleepy. Catheter passed an hour after nursing. About 1 ounce of very acid, Congo + fluid removed from stomach. Small amount of acid mucus obtained from duodenum.

December 26. Condition the same. Some bile tinged mucus aspirated from duodenum.

December 27. Condition worse. Increased jaundice. Some blood in stool, also subcutaneous hemorrhages. Rectal examination showed a considerable amount of dark fluid blood.

Catheter passed three-fourths of an hour after nursing. Congo +++ fluid obtained. About 0.3 c. c. of slightly bile-tinged fluid obtained.

December 28. Coagulation time of blood diminished (15 minutes). Temperature has ranged between 98 and 100 degrees. Weight 9 pounds 2 ounces; that is, only slight loss. Hemorrhages from bowel persist. An operation considered, but thought not advisable.

December 31. Subcutaneous injection of horse serum.

Duodenal catheter passed two hours after nursing. Congo ++ fluid aspirated from stomach, and in ten minutes about 1 c. c. of neutral fluid containing traces of bile obtained from duodenum.

January 2. Child drowsy. Hemorrhages the same. Taken away from hospital against advice.

The child was not seen again for about one month. On February 5 I was sent for and saw it at its home. Although it had lost weight it looked comparatively well and the hemorrhages had ceased. The child was being fed on condensed milk every three hours.

February 7. Doing poorly; has been vomiting; vomiting expulsive in nature. Stool still clay colored. Catheter passed; Congo +++ fluid evaculated from stomach. Marked cardiospasm, and great difficulty in entering stomach. (Probably food has not entered stomach.) Also difficulty in entering duodenum; "accessory method" has to be resorted to. Duodenal feeding with whey.

February 8. Still vomiting, as yesterday. Stool brown (starvation). Catheter passed. Cardiospasm the same, pylorospasm less. Duodenal feeding. Patient readmitted to hospital.

February 9. Doing poorly. Vomiting continuous. Cardia and pylorus less spastic. Duodenal feeding.

February 10. Exitus.

Clinically, the case was clearly one of congenital obliteration of the bile-ducts, showing intense jaundice, marked enlargement of the liver and spleen, and subcutaneous and intestinal hemorrhages. The urine contained a large amount of bile; the stools, although clay colored, also showed traces of bile. The duodenal catheter was frequently passed and determined the presence of bile in the duodenum, and also of the pancreatic ferments in the fluid which was aspirated. The infant did fairly well for three months and then suddenly developed vomiting which was followed by inanition and death. The cause of this vomiting could not be definitely ascertained. Possibly it was due to some error in feeding. It was markedly expulsive in nature, and characterized by cardiospasm, pylorospasm, and a hypersecretion and hyperacidity of the gastric juice.

An autopsy was performed by Dr. Eli Moschcowitz, whom I wish to thank for the following report:

AUTOPSY PROTOCOL

MACROSCOPIC

No. 2764. Heart and lungs not removed.

The liver weighs 170 gm. (formalin). Contour normal; consistency firm. Stained deeply bile-green. Surface roughened. On section the liver is deeply bile-stained; the cut surface shows green lobules with narrow, white spaces intervening. The lobules are slightly elevated; the white portions appear fibrous, and are situated at the periphery of the lobules in the portal spaces. The gross picture corresponds to that of a typical periportal cirrhosis. The remains of the ductus venosus enters the liver through a deep fossa hollowed out of the anterior border. There is a corresponding fissure on the under surface.

The fissure for the gall-bladder is at its normal site, and is occupied by a rather thick mass of fibrous tissue. In this mass there is a small

cavity (the gall bladder running longitudinally for a distance of about 2 cm. The lumen admits a narrow probe, and contains a few drops of clear watery fluid. The finest bristle passed from the gall-bladder in the direction of the cystic duct fails to find an exit.

The ducts of the liver are completely obliterated, and are represented by dense strands of fibrous tissue. The right and left hepatic ducts are impervious up to the point where they emerge from the liver, as well as the common hepatic duct.

The strand representing the common bile duct is somewhat thicker than the others, and passes to the posterior surface of the duodenum.

On the intestinal wall corresponding to the attachment of this strand is a small papilla within the lumen (to be described below).

The portal vein and the hepatic artery occupy their normal situations, and show no abnormality in size. There are a number of small lymph-nodes at the base of the fissure for the gall-bladder.

The duodenum is normally situated. Within the lumen and on the posterior aspect are two papillæ. The lower one, wider and deeper, is found to be the opening of a pancreatic duct, aand into this a fine bristle can be passed. The upper papilla is situated about 2 cm. distant and corresponds to the attachment of the fibrous remnant of the common duct, above described. This papilla is impermeable to the finest bristle.

The stomach, intestines, spleen, mesentery, kidneys, genito-urinary tract and the adrenals are normal; except for rather intense bile staining.

Anatomical Diagnosis: Complete obliteration of all the extra hepatic bile ducts and the papilla of Vater. Hypoplasia of the gall-bladder. Bile pigmentation of the liver, with periportal cirrhosis. Anomalous insertion of the ductus venosus. Anomalous position of accessory pancreatic duct (Santorini) below papilla of Vater.

MICROSCOPIC EXAMINATION

Liver: Under the low power the liver presents the typical appearance of rather advanced periportal cirrhosis. The connective tissue is abundant, loose, moderately infiltrated with round cells, and surrounds many of the lobules completely.

Interspersed in this connective tissue are abundant newly formed bile ducts, similar to those found in the cirrhosis of Laennec.

These ducts are very narrow, anastomose, and in any a few instances can a lumen be demonstrated; the lumen is empty.

Distinct transitions between the liver trabeculæ and the newly-formed bile ducts can be demonstrated in many instances.

The hepatic artery and the portal vein in the capsule of Glisson are normal, but no well formed bile-duct is present. There are nowhere evidences of cystic dilatations of the bile-ducts. The cells of the liver trabeculæ stain well; the nuclei are distinct and reticular. The intra- and intercellular bile-capillaries are everywhere dilated and filled with green bile. These dilatations are especially prominent in the central portions of the lobule. A noteworthy picture is the occasional appearance of a trabeculæ of the acinus glan type; the cells are arranged concentrically in a single row around a blood capillary. Such formations are few in number.

Section of the hypoplastic remnant of the gall-bladder reveals merely a dense fibrous structure, lined by a single layer of low cuboidal epithelium which is thrown up in low folds.

There is no structure corresponding to the submucosa or muscle fibers of the wall.

Section of the fibrous strands of the obliterated ducts shows nothing in any way suggestive of epithelium or lumen. These strands consist merely of dense fibrous tissue, with a few nerves and small blood-vessels.

Section of the upper papilla reveals this to be a small acessory pancreas in the wall of the duodenum. Serial sections reveal no evidence of a lumen, or of inflammatory tissue.

Sections of the other organs reveal nothing noteworthy. Pancreas normal; no cirrhosis.

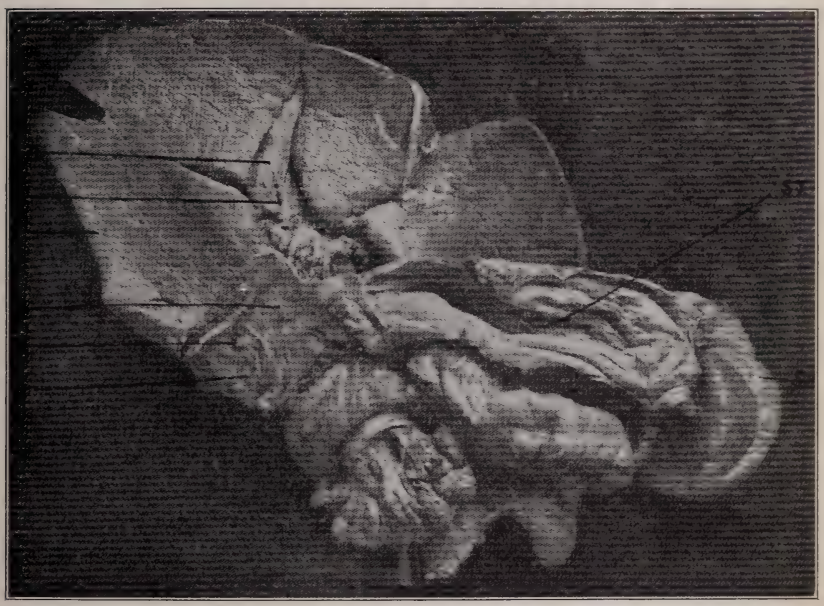


FIG. 2.—Obstruction of common bile-duct and pancreatic duct. *a*, accessory pancreas at site of papilla of Vater; *b*, opening of accessory pancreatic-duct (Santorini) below papilla of Vater; *c*, connective tissue representing common bile-duct; *g. b.*, rudimentary gall-bladder; *l*, liver; *st.*, stomach; *sp.*, spleen; *d*, duodenum.

DISCUSSION OF AUTOPSY REPORT

The autopsy therefore confirmed the clinical diagnosis of congenital obliteration of the bile ducts. All the extra hepatic ducts were found obliterated. The usual changes in the liver following marked stasis were present. The only notable features were the very exceptional site of entrance of the accessory pancreatic duct (Santorini) in the duodenum, which was below instead of above the papilla of Vater, and the accessory pancreas found in the wall of the duodenum at the site of the papilla of Vater (Fig. 2, a). On opening the duodenum this was seen as a small

nodule, resembling one of the solitary lymph-nodes commonly found in the lower intestine. Its true nature was not suspected. It was submitted to serial section with the purpose of ascertaining whether inflammatory tissue could be found at this point, as it is still an undecided question in the pathogenesis of this disease, whether the obliteration of the bile-ducts is due to a fetal inflammatory process, or to a developmental anomaly. No sign of inflammatory tissue was found on microscopic examination. The glandular arrangement of some of the liver strands, called attention to by Finkelstein,[4] is also worthy of mention.

Examination of Duodenal Fluid

In the clinical report mention has been made of examinations for pancreatic ferments carried out on the fluid aspirated from the duodenum. Quantitative and qualitative tests were made for the presence of trypsin, 5 per cent. gelatin being used for this purpose; for the tests for amylase a 1 per cent. starch solution was used, and after forty-eight hours the mixture was titrated for sugar by means of Fehling's solution; neutral ethyl butyrate was used to test for lipase, and after twenty-four hours the acidity was determined by means of titrating with twentieth-normal sodium hydroxid. All solutions were rendered alkaline by means of sodium hydroxid, and sterile by the addition of toluol. It does not seem necessary to describe these methods in detail, as an article soon to be published treats of a large series of tests made on infants by this means. It was found in this case that all three pancreatic ferments were being excreted into the duodenum. The trypsin was in considerable quantity as it digested gelatin within twenty-four hours. The amylase was likewise found not insufficient compared with the results obtained on normal infants. The lipase was, however, found to be diminished. Whether this was due to the lack of bile in the duodenum, which, as is well known, intensifies the action of the pancreatic fat-splitting ferment, or whether there was, in fact, a deficiency of lipase, it is difficult to say without further study. The stool contained a large amount of fat, as is the case in all instances of obstructive jaundice.

Cases with obliteration of the common bile-duct usually have been classified according to the obstruction of the hepatic ducts, the cystic duct, or the presence or absence of the gall-bladder. It would seem that this classification can possess no clinical or pathological significance where the common bile-duct is absolutely impervious. It would appear, however, by no means of purely academic interest to ascertain whether the pancreatic ducts are patent or likewise obstructed. In view of the fact that when the common duct is obstructed by a biliary calculus situated at the papilla of Vater, the pancreatic duct frequently is also obstructed, resulting in acute or chronic pancreatitis, it is remarkable that in the pathological condition which we are considering no attention whatsoever has been paid to the pancreas. In a rather extensive review of the literature I have not met with an instance in which the pancreas has been described at autopsy or later examined microscopically. For ex-

[4]Finkelstein, H.: Lehbuch d. Säuglingskrankh., 1912, Part II, p. 438.

ample, in Thomson's[5] excellent monograph, published in 1892, which carefully reviewed forty-nine cases, in Beneke's[6] report of 1907, which considered seventy-four cases, in reports of cases by Murchison,[7] Schueppel,[8] Henoch,[9] Rolleston,[10] and many others, no attention has been paid to the pancreas or its ducts. Indeed, the only mention which I have found on this subject was one by Wilks[11] in a report published in 1862, which states that "in the duodenum the opening of the bile-duct was found as usual and a probe passed freely into the pancreas." In almost all of these cases the obliteration of the common bile-duct included the papilla of Vater, which is known generally to harbor the outlet of the pancreatic duct as well as of the bile-duct. In these cases, unless there existed another duct from the pancreas to enable it to pour its ferments into the intestine, there must therefore have been in addition to an obstruction of the bile an obstruction of the external secretion of the pancreas. It is difficult to state in just what percentage of cases an accessory pancreatic duct, the duct of Santorini, enters the intestine separately. Opie[12] dissected 100 cases in order to investigate this subject and came to the practical conclusion that "in at least a third of all individuals the duct of Santorini cannot act as an accessory outlet when the duct of Wirsung is occluded."

Conclusions

In the pathologic condition termed congenital obliteration of the bile-ducts, the common bile-duct has been found almost invariably obliterated in the greater part of its course, including in almost every instance the duodenal end and the papilla of Vater. Into this papilla the main pancreatic duct almost always enters, and accordingly is subjected at the same time to absolute obstruction, so that it would seem preferable to term this disease congenital obliteration of the common bile-duct and pancreatic duct. A classification from a clinical point of view better than the purely anatomical one generally made use of would seem to be to divide this pathologic condition into two main groups of cases: (1) where associated with the obliteration of the common bile-duct there is an obliteration of pancreatic duct, which, however, is compensated for by the fact that the accessory duct, the duct of Santorini, is present and able to excrete the pancreatic ferments into the duodenum;[13] another group (2) where the obliteration of the excretory apparatus of the pancreas is total and complete, as not only is the duct of Wirsung absolutely obstructed, but no accessory pancreatic duct exists. Cases of congenital obliteration of the common bile-duct and pancreatic duct can, therefore, be divided into those with compensated or with uncompensated pancreatic

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[5]Thomson, J.: Congenital Obliteration of the Bile Ducts, 1892.
[6]Beneke, R.: Kongenitale Atresia d. grossen Gallengänge, Marburg, 1907.
[7]Murchison: Diseases of the Liver, 1885, p. 422.
[8]Schueppel: Arch. f. Heilk., 1870, p. 78.
[9]Henoch, E.: New Syd. Soc. Tr., i, 28.
[10]Rolleston, H.: Diseases of the Liver, 1905.
[11]Wilks: Tr. Path. Soc. London, 1862, p. 119.
[12]Opie, E.: Am. Med., 1903, p. 996.
[13]Under this group would come those exceptional cases in which the common bile-duct and the duct of Wirsung enter the duodenum separately and at different levels.

obstruction. The case described in the foregoing pages was of the compensated variety, which comprises the larger group. During life the three pancreatic ferments, trypsin, amylase, and lipase, were repeatedly found in the duodenal fluid obtained by means of aspiration with the duodenal catheter, and the diagnosis of compensated pancreatic obstruction was substantiated after death by finding the main pancreatic duct obliterated and the accessory duct patent.

It is of interest to note that notwithstanding the absolute closure of the common bile-duct, bile was demonstrated in the duodenal fluid and in the stool, proving the probable excretion of bile from the circulation through the intestinal wall. The infant lived for three months, and died as the result of inanition brought on by an acute attack of vomiting accompanied, as tests by means of the catheter showed, by cardiospasm, pylorospasm, and hyperacidity and hypersecretion of the gastric juice. This complication may bear no relation to the underlying pathologic disturbance; reference to the literature in regard to this point, however, shows that marked vomiting has been an important intercurrent symptom in many cases, so that attention should be directed to this complication in the future.

It is probable that it will be found that the cases of congenital obliteration of the bile-ducts associated with uncompensated obstruction of the pancreatic ducts are the more serious, and that infants in this group survive for a shorter period than those in whom the ferments gain entrance to the duodenum by an accessory duct. A test of all cases by means of the duodenal catheter will permit of a clinical classification during life of the compensated and the uncompensated cases, and the data obtained by this means should be carefully correlated with the results of post-mortem examination, especially with the pathological condition of the ducts and parenchyma of the pancreas, an organ which has been absolutely disregarded in the consideration of this disease.

ATTEMPTS TO PRODUCE SCARLATINA IN MONKEYS

CHARLES KRUMWIEDE, JR., MATTHIAS NICOLL, JR., AND JOSEPHINE S. PRATT

Notwitstanding the numerous attempts to inoculate the lower monkeys with scarlet fever, the positive results reported, notably those of Cantacuzene and Bernhardt, have not been substantiated by others who have repeated their experiments. Thus Landsteiner and Levaditi inoculated thirty-five lower monkeys of five different species with exudate from the throat and with the blood and lymph-nodes of scarlet fever patients by local application to the animals' fauces and by intravenous, intraperitoneal and subcutaneous injections of scarlet fever products without having obtained a seemingly positive result. Klimemko, making use of twenty lower monkeys of five species, was equally unsuccessful, although he included among his many experimental procedures that of Bernhardt, who claims to have produced the disease in fourteen of nineteen animals. Shortly after the publication of Cantacuzene's first papers, Dr. Park inoculated six monkeys with the blood of six fresh scarlet fever cases, including several of the malignant type. The technique employed was the same as described in Cantacuzene's report. The results* were completely negative. Our few experiments are herewith given as an additional contribution to the proof that this class of monkey is immune to the virus of scarlet fever when inoculated by any method heretofore made use of.

Some of the inoculations recorded had their inception in a laboratory accident. An assistant while preparing the suspensions of streptococci for immunization of horses sucked some of the suspension into her mouth. This accident occurred on October 24, 1912. On October 26th, a. m., a beginning sore throat was noticed which became progressively worse during the day. The sore throat with general symptoms of prostration continued and on October 28th, tingling of the skin of the arms and chest was noticed, probably the beginning of the exanthem. When seen Tuesday a. m. a general pharyngitis was present, and a typical scarlatinal rash covering the arms, chest and abdomen, the legs being comparatively free. The temperature was 101.6 degrees F., rising to 102 degrees later in the day. During the next two days the temperature fell to normal and the throat and general symptoms disappeared. The subsequent course was uneventful, the desquamation taking place in a typical manner. As far as could be determined, there had been no contact with a case of scarlet fever.

The streptococci in the suspension were as follows:

Scarlet fever—throat, 4 strains, 2 haemolysing, 2 green.
Mastoiditis, 3 strains, haemolysing.
Puerperal sepsis, 3 strains, haemolysing.
Pleurisy, 1 strain, green.
Erysipelas, 1 strain, haemolysing.

* Unpublished.

No. 593. *Java, Female—*

November 25, 1912. Throat swabbed and sprayed with a heavy suspension of the strains above mentioned.

Observations—

Temperature—This was taken ten days before the animal was used to determine the normal range. It fluctuated between 100 degrees and 101.4 degrees F. Subsequent to inoculation the temperature range remained the same, except for one rise to 102.2 on November 28.

Throat and Tongue—No change observed. Blood counts—see table.

No inclusion bodies (Döhle) were seen in any of the smears.

Dog Faced Baboon. A. Male—

December 3, 1912—Throat swabbed and sprayed with suspension of the same strains.

Observations—

Temperature—For eight days preceding inoculation fluctuated between 99.4 and 101.6 degrees F. Subsequent to inoculation the highest point was 101.8 degrees.

Throat and Tongue—No changes observed. Blood counts—see table.

No inclusion bodies (Döhle) were seen in any of the smears.

Dog Faced Baboon. B. Female—

December 13, 1912—Throat swabbed and sprayed with the same strains.

Observations—

Temperature was taken only three days before injection and ranged about 101 degrees F. Subsequent to injection it rose at times to 102 degrees F. and twice to 102.2 degrees F.

Throat and Tongue—No changes noted. Blood counts—see table.

Inclusion bodies—small dot-like bodies were occasionally noted. They were slightly more numerous on December 4.

Conclusions—

Mixed streptococci from scarlet fever and other sources which have been grown for some months on artificial media cause no perceptible reaction when applied to the throat of monkeys.

No. 593. *Macacus, Female—*

December 6, 1912—Two cubic centimeters of sterile 10 per cent. sodium citrate solution was drawn into a syringe. The syringe was then used to take 25 c. c. of blood from a child with scarlet fever. The case was four and one-half days ill, showing a profuse rash over body, typical scarlatinal tongue and throat, and enlargement of the cervical inguinal and axillary lmypth nodes. The temperature at the time was 102 degrees F. The blood was injected intarperitoneally into the monkey within a few minutes after withdrawal.

Observations—

Temperature—Before and after injection the range was the same, fluctuating between 101 degrees F. and 102.2 degrees F. On December

10, 11, 12 and 13 the temperature ranged from 102 degrees to 102.2 degrees F. At no other time did the temperature remain at this height.

Blood counts—see table. No inclusion bodies seen.

Throat and Tongue—No change observed.

The noteworthy points in this experiment are that on the day after the injection the temperature reached its highest point and remained high for four days. On the day preceding this rise the polynuclear percentage rose and during the four days of highest temperature the total count rose as well as the polynuclear percentage. As the monkey was menstruating at this time no weight can be placed on these changes. At best they were too slight to be even suggestive of a successful inoculation.

Dog Faced Baboon A—

December 13, 1912—Twenty-five cubic centimeters of blood were taken from a child thirteen years old, five days ill with scarlatina. A typical rash and scarlatinal throat and tongue were present. The temperature at the time was 100 degrees F. The blood was injected intraperitoneally immediately after withdrawal.

Observations—

The temperature reached 101.8 degrees before injection but fluctuated as a rule closely around 101 degrees F. After injection the range was between 101 degrees and 102 degrees F., giving in the chart the appearance of a slight transient rise.

Throat and Tongue—No changes observed. Blood counts—see table. No inclusions found in any of the smears.

Results—

The intraperitoneal injection of 25 c.c. of blood into a macacus monkey was followed by slight rise in temperature and a coincident increase in the total and polynuclear leucocyte count, this rise, however, was slight and probably due to other causes. A like injection into a baboon was followed by no reaction.

Dog Faced Baboon. 12. Female—

March 31, 1913—Swabs were smeared with the discharge from ears, throat and nose of seven children, ill from four to fifty-five days with scarlet fever alone or with scarlet fever and subsequent complications. The material on the swabs was washed off in sterile normal saline solution. This suspension was filtered through a Bekefeld filter, throwing the first filtrate back on the filter and passing saline through the filter after the suspension has passed through. Forty cubic centimeters of filtrate was obtained. This filtrate was bacterially sterile. This was injected intraperitoneally.

Observations—

Temperature—This rose to 102 degrees F. the second day after injection. The normal range for this animal was about 101 degrees F., occasionally rising a half degree higher.

Throat and Tongue—No changes noted.

Dog Faced Baboon. 12. Female—

April 21, 1913—Swabs were collected as in the preceding experiment These were washed off in saline giving a total of 4 c. c. of suspension. This was sprayed and swabbed on the animal's throat.

Observations—

Temperature—Did not rise above 101.6 degrees F.

Throat and Tongue—No change.

Results—

The intraperitoneal injection of bacteria free material from scarlet fever cases, and the direct application to throat of the same type of material without filtration were followed by no reaction.

Summary—

The attempt to produce scarlet fever in monkeys and baboons by the faucial inoculation of streptococcus cultures or unfiltered exudates and discharges from scarlet fever cases, as well as the intraperitoneal injections of filtrates of such exudates and discharges, and of large quantities of blood, gave negative results.

Leucocytes—Total Number and Percentage of Polynuclears

Animal		593		A		B		593*		F*	
Before Inoculation—Day	8	—	—	12,600	43%	—	—	—	—	—	—
	7	—	—	15,600	27%	—	—	—	—	11,500	38%
	6	13,850	59%	13,150	40%	—	—	—	—	—	—
	5	10,500	67%	—	—	—	—	—	—	—	—
	4	12,000	63%	11,000	33%	—	—	10,500	57%	10,500	44%
	3	12,500	64%	—	—	—	—	—	—	—	—
	2	13,000	65%	—	—	11,000	48%	10,700	53%	—	—
	1	10,500	64%	14,000	40%	14,000	35%	—	—	—	—
	0	13,300	68%	—	—	—	—	—	—	—	—
After Inoculation—Day	1	13,600	59%	12,400	36%	12,500	54%	11,300	56%	13,000	43%
	2	11,300	66%	12,000	42%	—	—	—	—	—	—
	3	—	—	—	—	13,000	46%	12,500	70%	9,000	54%
	4	11,200	62%	11,500	38%	11,000	50%	16,500	68%	9,000	37%
	5	—	—	—	—	13,000	33%	14,000	70%	10,400	31%
	6	—	—	—	—	—	—	10,500	61%	—	—
	7	10,500	57%	10,500	44%	—	—	12,500	68%	—	—
	8	—	—	—	—	10,200	—	9,000	59%	9,100	—
	9	10,700	53%	—	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
	11	—	—	—	—	—	—	9,300	52%	—	—
	12	—	—	—	—	—	—	—	—	—	—
	13	—	—	—	—	—	—	—	—	—	—
	14	—	—	—	—	—	—	—	—	—	—
	15	—	—	—	—	—	—	10,300	—	—	—

* Second inoculation.

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A STUDY OF THE VIRUS OF RABIES, FREED FROM THE CELLS OF THE HOST AND FROM CONTAMINATING ORGANISMS

Daniel W. Poor and Edna Steinhardt

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I.—Introduction

Experimental work on rabies has largely been carried on with the virus as it occurs in the brain and spinal cord, where it is closely associated with the elements of the nerve tissue. Comparatively little has been done with the virus as it occurs in the salivary glands, where it is presumably in a more or less free state.

By the application of two new methods [1] we have been able to obtain

[1] Poor and Steinhardt, Jour. Infect. Dis., 1913, 12, p. 202.

a virus of rabies from the submaxillary glands in a comparatively pure state, freed from cells of the glands and unmixed with other organisms. Our work with this virus has been carried on with two objects in view: (1) the determination, if possible, of its nature, and (2) a comparison of this gland virus with the brain and cord virus.

If this comparison brought out marked physical differences, it seemed to us that this would suggest that the organism undergoes a cycle and exists in a different condition in the submaxillary gland from that in which it is found in the nervous system.

Of course, we have also used our pure virus for microscopical and cultural studies, but although considerable work has been done in this direction, the results as yet have been inconclusive, and we are still continuing the experiments.

II.—Experimental Work

Methods of Obtaining the Virus from the Submaxillary Glands.

GLYCERIN EXTRACT VIRUS

The submaxillary glands from dogs, dead of street rabies, were used in both methods. Several years ago one of us[2] found that the virus of rabies could be extracted with glycerin from the submaxillary glands of rabid dogs.

The glands were removed from the dog's head under aseptic precautions. After removal of the fibrous sheath, they were immersed in sterile neutral glycerin and allowed to stand in the icebox for six to twelve days. This glycerin extract retained its virulence when kept in the icebox for a very long time, in one experiment 191 days. No further work was done on the extract due to the limitations of experimenting with a glycerin virus.

In our present experiments, we found that although virus may be extracted from the intact glands, more was obtained when the glands were cut in eight to ten pieces, and this was always done in the following work. The clear supernatant glycerin slightly colored by hemoglobin was pipetted off. This was usually found to be uncontaminated, and microscopical examination showed no formed cellular elements.

By our present method, dialysis through collodion sacs, we are able to get rid of glycerin and have our virus uncontaminated in any fluid we select. All the operations are carried on under aseptic conditions. The sac is sterilized, the glycerin extract contains no contaminating organisms, and as the collodion membrane does not permit the usual bacteria to pass through, the fluid in which the sac is placed for dialysis need not be sterile.

The dialysis of the glycerin takes place very rapidly. In running physiological salt solution, Ringer's solution, or distilled water, one and one-half hours are usually sufficient for the removal of the glycerin from four or five cubic centimeters of the virus. After dialysis, the virus is

[2]Poor, Proc. New York Path. Soc., 1906, 6, p. 85.

diluted, the dilution varying with the amount of water the glycerin has previously extracted from the tissue.

If a concentration is desired, the collodion sac may be used as a filter, as according to our experiments this virus does not pass through it, and, therefore, as much of the fluid as is desired may be removed. It is possible that a certain amount of the virus adheres to the side of the sac, so the concentration cannot be computed exactly. As shown by one of us,[3] proteins and probably salts in solution gradually pass through the sac after absorption reaches a certain degree, but the protein and salt contents of the fluid within the sac will be somewhat concentrated as the water passes through most rapidly. This fact must be borne in mind in interpreting some of the results of concentration.

The technic used for making collodion sacs was that described by Novy[4] and modified by Gorsline.[5] The sacs were made over a tube, which had a small opening in the bottom. This tube was turned several times in dilute collodion, and this collodion, when partially dried, was stripped off by forcing water through the tube. A glass tube was then inserted into the neck of the sac, and the junction strengthened by a ligature or coating of collodion.

The sacs were filled with distilled water, the tubes plugged with cotton, autoclaved at 105° C. for fiften minutes, allowed to cool, then emptied, immersed in sterile water and subjected to pressure (about three inches of mercury). If there was no evidence of leakage the sacs were used. The sacs were without flaws or air bubbles. For dialysis they were thick; for filtration thin. The virus obtained in this manner will be refered to as the *glycerin virus.*

THE ASPIRATION VIRUS

The glands after being cut in eight to ten pieces were placed in distilled water and subjected to a vacuum of 29 inches of mercury for an hour. The pieces of glands were then pressed in an ordinary meat press and the expressed fluid centrifuged. After centrifugation the supernatant fluid was pipetted off and added to the water in which the glands were aspirated. The aspiration fluid alone was found to contain the virus, but more was present on the addition of the pressed-out juice. By this method a suspension of the virus was obtained in distilled water which was stronger than the glycerin virus, but which was not sterile and which contained fragments of gland cells, blood cells, etc. Subsequent experiments showed that a Berkefeld filter held back bacteria and formed tissue elements, but the virus passed through. Thus by filtration we were able to get a sterile virus. This virus will be referred to as the *aspiration virus.*

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[3]Steinhardt, Jour. Infect. Dis., 1910, 7, p. 675.
[4]Laboratory Work in Bacteriology, Ann Arbor, 1899, p. 499.
[5]Contrib. Med. Res., Ann Arbor, Mich., 1903, p. 390.

TABLE 1

VIRULENCE TESTS OF ASPIRATION GLAND VIRUS (STREET RABIES)

Number of Test	Guinea-Pigs Inoculated	Number Infected	Individual Incubation	Average Incubation
			Days	Days
1	3	3	8 9 8	8 1-3
2	2	2	8 10	9
3	2	2	9 9	9
4 (Two sets of glands aspirated together)	3	3	9 8 8	8 1-3
5 (Four sets of glands aspirated together)		2	8 8	8
6 (Two sets of glands aspirated together)	2	2	10 10	10
7 (Three sets of glands aspirated together)	3	3	16 19 25	20

VIRULENCE TESTS OF THE GLYCERIN EXTRACT VIRUS

Glycerin extracts of the submaxillary glands from 45 rabid dogs (street virus) were tested for virulence. The brains of most of these dogs showed Negri bodies. As Table 2 shows, 33 of the extracts were virulent or 73.33 per cent.; the inoculated guinea-pigs developed rabies with incubation periods ranging from eight to eighty-two days. Of the non-virulent extracts the longest period in which the animals were kept was sixty-nine days. This is unfortunate, as we now realize that had we kept the animals longer, more cases of rabies might have developed.

In a few cases where the smears of the fresh brain showed no positive Negri bodies, the gland extract, either gylcerin or aspiration virus, proved virulent with a short incubation period, shorter than the brain inoculation. Therefore, occasionally this virus may be used for diagnostic methods, especially in those cases in which the brain is not fresh, and with the aspiration virus of the glands the Berkefield filtrate can be used.

The glycerin extract was diluted with twice the amount of salt solution and about one-third of a cubic centimeter was inoculated subdurally into each guinea pig. Although the guinea pigs showed clinical rabies, the brain smears were always examined for Negri bodies and if not found reinoculations were made into fresh guinea pigs. The possibility of a contaminating infection giving paralytic symptoms closely resembling rabies was brought to our notice in some of our animals and a report of that work will be published later.

TABLE 2

VIRULENCE TESTS OF GLYCERIN EXTRACTS OF SUBMAXILLARY GLANDS OF RABID DOGS (STREET VIRUS)

Virulent Extracts

Number of Test	Number of Days in Glycerin	Number of Guinea-Pigs Inoculated	Number Infected	Individual Incubation Period (Onset of Symptoms)	Average Incubation Period
				Days	Days
1.....	14	3	3	8 13 14	11 2-3
2.....	16	2	2	11 11	11
3.....	10	3	2 (1 died 24 hrs. after inoculation; not rabies)	10 11	10 1-2
4.....	4	3	3	14 10 10	11 1-3
5.....	10	3	3	16 18 18	17 1-3
6.....	4	3	3	10 10 10	10
7.....	10	3	3	10 12 14	12
8.....	13	3	2 (1 died in 4 days; not rabies)	13 13	13
9.....	6	3	2 (1 died in 7 days; not rabies)	55 65	60
10.....	6	3	2 (dead in 6 days; not rabies)	13 13	13
11.....	8	3	3	7 16 17	13 1-3
12.....	8	3	3	9 11 11	10 1-3
13.....	5	3	3	13 13 22	16
14.....	6	3	2 (1 discharged)	15 21	18
15.....	6	3	3	21 21 21	21
16.....	7	3	1 (1 dead in 1 day; 1 dead in 6 days; not rabies)	82	82
17.....	7	4	1 (2 dead in 4 days; not rabies. 1 discharged after 82 days)	34	34
18.....	7	3	2 (1 dead in 4 days; not rabies)	12 20	16

TABLE 2—*Continued*

Number of Test	Number of Days in Glycerin	Number of Guinea-Pigs Inoculated	Number Infected	Individual Incubation Period (Onset of Symptoms)	Average Incubation Period
19.....	7	3	1 (2 dead in 4 days; not rabies)	12	12
20.....	7	3	3	18 18 19	18 1-3
21.....	7	3	2 (1 discharged)	13 13	13
22.....	7	3	1 (1 dead in 4 days; 1 dead in 5 days; not rabies)	15	
23.....	13	2	1 (1 dead in 5 days; not rabies)	14	14
24.....	6	3	1 (1 dead in 2 days; 1 dead in 4 days; not rabies)	16	16
25.....	8	3	2 (1 dead in 24 days; not rabies)	10 12	16
26.....	6	2	1 (discharged in 180 days)	21	21
27.....	7	3	2 (1 discharged)	10 10	10
28.....	7	3	1 (1 dead in 25 days; not rabies. 1 discharged)	11	11
29.....	8	3	2 (1 discharged)	17 18	17 1-2
30.....	12	3	1 (2 discharged after 138 days)	14	14
31.....	14	3	2 (1 discharged)	9 10	9 1-2
32.....	12	3	1 (1 missing after 4 days. 1 discharged 36 days)	18	18
33.....	14	3	1 (2 missing after 6 days)	18	18

Non-Virulent Extracts

Number of Test	Number of Days in Glycerin	Number of Guinea-Pigs Inoculated	Number Infected	Date Discharged
				Days
1.....	50	2	None	37
2.....	23	2	None (1 dead in 18 days. No Negri bodies found. Reinoculation negative)	37
3.....	14	3	None	60
4.....	4	3	"	53
5.....	14	3	"	47
6.....	7	3	"	47
7.....	13	3	"	47
8.....	14	2	"	69
9.....	8	3	"	2 discharged in 85 days
10.....	6	3	" (1 died in 64 days; not rabies)	2 discharged in 85 days
11.....	10	3	None (1 dead in 26 days; not rabies)	2 discharged in 138 days
12.....	14	3	None	172

That a single glycerin extraction may not remove all the virus from the glands was shown by the following experiment. In the virulent extracts, the glycerin extract of Virus 15 was withdrawn from the glands after six days and fresh glycerin added. This second glycerin extract was removed after eight days. The first extract gave rabies to guinea pigs (3 in each set) with an average incubation period of twenty-one days, while the second gave rabies with an average of twenty-four and two-thirds days. This showed but a slight dilution of the virus.

Virulence Tests of Submaxillary Glands Whose Glycerin Extracts are Negative

That the action of glycerin on the glands is mainly that of sucking out the virus from the tubules is seen from the following experiment. The glands from two rabid dogs had given a non-virulent glycerin extract. They were removed from the glycerin and ground up in a mortar with salt solution, a difficult operation owing to the tough and slippery condition of the glands. These emulsions, extracted in the icebox overnight, proved to be virulent, giving rabies with an average incubation period of twenty-nine days in one case, and of twelve days in the other. In another set of glands in which the glycerin extract had an incubation period of fifty-six days, the ground-up glands gave rabies in ten days.

On the other hand, in some glands much virus was present in the ducts, as we have shown by a second extraction of virus with fresh glycerin giving rabies with a short incubation, confirming the fact of the variability of the virulence of the saliva of rabid animals.

Virulence Tests of Glycerin Extracts of Other Organs

Rabid Brains.—Glycerin extracts were made of 11 fixed virus brains (rabbit) of which only four were virulent, and the glycerin extracts of four street virus brains (dog) were all non-virulent (see Table 3), showing in all only four virulent extracts from 15 brains—that is, 26.66 per cent. virulence compared with 73.33 per cent. of the glycerin gland extract.

When one considers that the brain is the culture medium for the rabies organism, which is present in much greater numbers in this situation than in the glands, one would expect a greater number of virulent brain extracts, unless, as is undoubtedly the case, the virus is here intimately associated with the nerve cells. Indeed the virulent extracts from the brain virus might be accounted for by minute particles of the brain substance being torn off by the action of the glycerin (the brain being a more delicate tissue than the gland) or by the extraction of the virus from the blood and lymphatic vessels and spaces.

In one case where the brain of a rabid dog (street virus) showed Negri bodies, part of the Ammon's horn, suprarenal capsule, and sciatic nerve were immersed in glycerin; the extracts were tested for rabies and all were non-virulent. In three cases of street rabies the glycerin extract of the pancreas was non-virulent.

These experiments are too few from which to draw conclusions; they were carried out to try the glycerin extraction on other organs which have been found virulent in rabies. Whether the aspiration method would have shown the virus to be in an extractable condition in these organs is to be decided by further experiments.

TABLE 3

VIRULENCE TESTS OF GLYCERIN EXTRACTS, RABID BRAINS OF RABBIT FIXED VIRUS AND DOG STREET VIRUS

No. of Test	Material Extracted	Number of Days in Glycerin	Filtered	Guinea-Pigs Inoculated	Number Infected	Average Incubation Period	Discharged
		Days					Days
1...	Fixed virus rabbit brain	9	Not filtered	2	None	None	36
2...	" " " "	12	Not filtered	3	"	"	53
3...	" " " "	12	Through cotton	3	"	"	53
4...	" " " "	9	a Not filtered	3	2	11½ days	1 in 46
			b Through 1 layer of filter paper	3	1	13 "	2 in 41
5...	" " " "	9	a Not filtered	3	2	9 "	1 in 46
			b Through 1 layer of filter paper	3	2	10½ "	1 in 41
6...	" " " "	11	Through 1 layer of hardened filter paper	3	2	8 "	1 in 46
7...	" " " "	11	Through 1 layer of hardened paper paper	3	None	.....	46
8...	" " " "	10	Through 1 layer of hardened filter paper	3	1	8 "	"
9...	" " " "	10	Through 1 layer of filter paper	3	None	.....	"
10...	" " " "	10	Through 1 layer of filter paper	3	"	.....	"
11...	" " " "	10	Through 1 layer of filter paper	3	"	.....	"
12...	Street virus (dog) brain	16	Through cotton	3	"	.....	33
13...	" " " "	16	Through cotton	3	"	.....	"
14...	" " " "	16	Through 1 layer of filter paper	3	"	.....	"
15...	" " " "	12	Through 1 layer of filter paper	3	"	.....	"

COMPARISON OF THE EFFECT OF GLYCERIN ON DIPHTHERIA AND TUBERCLE BACILLI

Emphasis is usually laid on the resistance of the parasite of rabies to the action of glycerin, and this resistance is frequently considered to be opposed to the virus being of a bacterial nature.

We have made comparative tests with cultures of tubercle and diphtheria bacilli. Ten cubic centimeters of pure sterile glycerin were put on the cultures (agar and egg media) so that the growth was completely immersed. These were placed in the icebox for two weeks in the dark.

The supernatant glycerin from the diphtheria culture was dialyzed and planted in broth; incubation at 37.5° C. gave a vigorous growth of diptheria organisms. The examination of the stained slides showed the bacilli in glycerin to be perfectly stained although somewhat small. After

dialysis, the stained slides from the non-incubated fluid showed bacilli indistinguishable from those from a fresh culture.

On cultures of bovine tubercle bacilli on egg media about 10 c.c. of glycerin was added so that the growth was more than covered and placed in the icebox in the dark. Three weeks later the supernatant glycerin was clear; there were no visible particles in it. Three cubic centimeters of this glycerin were dialyzed in running 0.8 per cent. salt solution in a collodion sac for two hours. This dialyzed fluid was inoculated into guinea-pigs and produced general tuberculosis in two to three weeks.

After an interval of 13 months in the icebox, a portion of the growth of the same cultures, still covered by glycerin, was removed, emulsified and injected into guinea-pigs. These died of general tuberculosis, showing that the tubercle bacilli had not lost their virulence even after being acted upon by the glycerin for 13 months. The staining showed no diminution in the acidfastness of the organisms, and transfers gave growth on egg medium.

These two cultures, diphtheria and tubercle bacilli, are organisms which are not killed by drying, and as the action of glycerin is largely the withdrawal of water from that with which it is in contact, our results are not surprising. We find that these bacteria are not destroyed by glycerin, the tubercle bacillus having a resistance as great as the virus of rabies. This resistance, therefore, cannot be considered as necessarily indicating a protozoal virus.

We are now starting experiments on the action of glycerin in the cold and dark on other organisms, that may possibly have some bearing on the virus of rabies.

Comparison of Brain and Gland Virus

There are various hypotheses as to the nature of the virus of rabies. Some investigators consider it of bacterial origin with possible spore formation, while others favor a protozoal theory with a definite cycle. There are no Negri bodies in the gland virus though they exist in the brain. To test the question of a possible difference in the physical state of the virus in the two situations, brain and gland, differences which might throw light on the supposed cycle or spore formation, comparative experiments were made on viability, virulence, action of glycerin, the effect of rapid drying, of heat, of combined drying and heat, of dialysis, and of some chemical agents. In no instance was there any definite difference in the reaction of the two viruses to any of the tests, showing that as far as our work goes at present, it all indicates a similar physical condition of the virus of rabies in the gland and in the brain.

Viability.—Gland virus, aspirated in distilled water, was kept in an icebox at 7-8° C. for 15 days, at the end of which time it had become offensive through bacterial contamination. It was then filtered through a Berkefeld V candle, a filtrate being obtained which produced rabies in guinea-pigs with an incubation of 14 days. This filtrate kept at room temperature and exposed to light for 45 hours had lost its virulence completely.

An emulsion of fixed virus in distilled water exposed to the same conditions for the same length of time was markedly weakened, the incubation of the injected animals being increased to 10 days, five days longer than the controls. Continuing the exposure to light and room temperature for 96 hours destroyed the virulence of the brain emulsion.

This experiment gives but a rough comparison as there were undoubtedly many more organisms present in the fixed virus emulsion than in the gland virus filtrate, and, as is known, the number of organisms present, and the amount of protein and salts in the emulsion greatly modifying the results.

In another experiment the Berkefeld filtrate from an aspiration gland virus kept in the icebox for nine days had lost its virulence while the unfiltered virus kept in the same manner for the same length of time and then filtered proved virulent. This is in accord with the experiments of Di Vestea[1] on brain virus. He found that the filtrate of fixed virus died out in the icebox within a week, while the original unfiltered virus retained its strength for a long time.

Di Vestea also found the filtrate to be more easily destroyed by heat and glycerin, and from these results he concludes that the filtrate does not contain the entire rabic virus, that the virus is of a protozoal nature, of which only the young forms are filterable, and that these young forms are more easily destroyed by heat and glycerin and conservation. Di Vestea's facts are undoubtedly correct, but there is another and simpler interpretation for them. As we have said before, in all comparative tests, variations in the number of organisms present and the concentration of proteins and salts of the material tested vary the results greatly. There are undoubtedly fewer organisms in a filtrate than in the original unfiltered virus and as shown by one[2] of us unless one filters a sufficient amount to saturate or coat the filter, the filtrate will contain considerably less proteins than the original solution. The fact that Di Vestea found his filtrates less resistant than his original emulsion and the confirmation of that fact in this work with gland virus is more probably explained by the fewer number of organisms and the lesser amount of proteins in the filtrate than by a protozoal cycle.

Comparative virulence.—The incubation period of the gland virus is usually much shorter than that of the brain in street rabies. Subdural inoculation in guinea-pigs with emulsion of dog's brain, street virus, generally produce rabies with an incubation period of 14 days, as compared with the usual eight to nine days with the gland virus. This difference in incubation period could be explained in several ways: (1) the lesser virulence of organisms in the brain virus; (2) the lesser number of organisms in the brain virus; (3) the presence of brain substances in the inoculations with this virus; this brain substance might act either directly or by causing a slowness of absorption.

One experiment with the glycerin extract of a dog's brain, street virus, showed an incubation period of only nine days. As in our other

[1]Ann d'ig. sper., 1905, 15, p. 147; La Med. Ital., 1904, 2, p. 241.
[2]Steinhardt, Jour. Med. Research, 1904, 13, p. 409.

glycerin extract tests a piece of brain of a rabid dog, street virus, was put in glycerin for six days and then some of the supernatant glycerin was removed. This glycerin was not filtered and contained many small particles of brain matter, so we could not consider it as an extract and omitted it from Table 3. Three guinea-pigs were inoculated with it using the same methods and dilutions as for the other extracts. Of these, one contracted rabies with an incubation of nine days, the other two were negative and discharged 108 days later. Thus at least for this one case the street brain was as virulent as the gland extract. As yet we have not made a further study of this question and until more work has been done, no statement can be made as to the comparative strength of the street virus in the two situations.

The fixed virus is undoubtedly the stronger, judging from the shorter incubation period, even at great dilutions.

Action of glycerin.—The action of glycerin, according to Marie,[1] in destroying the virus of the rabid brain is a slow one if the mixture is kept at icebox temperature and is dependent on the amount of brain tissue, and it is difficult to fix the limits of this action.

Poor found, as quoted before, that under similar conditions the gland extract retained in virulence a long time. In one case at the end of 191 days, guinea-pigs inoculated with the glycerin extract showed rabies after 12 days' incubation.

In the present experiments, we have found a gradual weakening of the glycerin gland virus, kept in the icebox. This weakness was shown by a prolonged incubation period or complete avirulence.

In one instance, the glycerin that had been on the glands for 10 days was inoculated into three guinea-pigs. It infected all with an average incubation period of 12 days. The same extract kept on the gland in the icebox, 37 days later infected two out of three guinea-pigs with an average incubation of 19 days.

In one case we tested a virulent glycerin extract virus after six months' preservation in the icebox and found it had lost its virulence.

When kept in the icebox, the action of glycerin on the virus of rabies in the brain or in the gland, although a variable one, brought out no difference between the virus from these two sources.

Rapid drying.—It is an established fact that brain virus if dried rapidly retains its virulence. That gland virus under similar conditions is equally as resistant is shown by the following comparative experiment.

As Harris and Schackell[2] state that it is the concentration of salts in the drying which causes the destruction of the rabies virus, to partially eliminate this factor, a preliminary dialysis in distilled water was done with the brain virus. An emulsion of fixed brain virus was made with distilled water put in a collodion sac and dialyzed in running distilled water for two hours. Part of this dialyzed fluid was kept in the icebox as control. The remainder was spread in a thin layer and dried in a vacuum of 29 inches of mercury over sulfuric acid. After 16 hours the

[1]L'étude expérimentale de la rage (Dorin et Fils, editors), 1909, p. 124.
[2]Jour. Infect. Dis., 1911, 8, p. 47.

drying was complete and the material was rubbed up in salt solution to its original volume. Subdural inoculations in guinea-pigs with the same doses of this and the control emulsion produced rabies in five to six days in both cases.

The glands from two rabid dogs (street virus) were aspirated in distilled water separately. These two aspiration gland viruses were put under the same conditions as the brain virus.

The results here also showed no destruction of the virus, as inoculations with both the dried and the control gland virus gave rabies to guinea-pigs in nine and one-half days.

TABLE 4

EFFECT OF RAPID DRYING ON EMULSIONS OF FIXED BRAIN VIRUS AND ASPIRATION GLAND VIRUS (STREET)

Number of Test	Material Inoculated	Guinea-Pigs Inoculated	Individual Incubation	Average Incubation
			Days	Days
1 (Control fixed virus).....	Fixed virus brain emulsified in distilled water and dialyzed	3	5 5 7	5 2-3
2 (No. 1 dried)....	5 c.c. of No. 1 dried in vacuum, then emulsified to original volume with salt solution	3	5 5 7	5 2-3
3 (Control fixed virus).....	Fixed virus brain emulsified in salt solution	2	5 5	5
4 (No. 3 dried)....	5 c.c. of No. 3 dried in vacuum, then emulsified to original volume with distilled water	2	1 dead in 7	7
5 (Control gland virus A).....	*Aspiration gland virus (street) A	2	8 10	9
6 (No. 5 dried)....	Same as gland virus A. Dried in vacuum for 10–15 hours, then emulsified with salt solution	3	9 13 10	10 2-3
7 (Control gland virus B).....	*Aspiration gland virus (street) B	3	9 9 10	9 1-3
8 (No. 7 dried)....	Same as gland virus B. Dried in vacuum for 10–13 hours, then emulsified with salt solution	3	10 8 9	9

* In distilled water.

Effect of combined rapid drying and heat.—An emulsion of fixed virus and a glycerin gland virus was dialyzed in distilled water for an hour. Very thin layers of each were evaporated to dryness while exposed to 37° C. for six hours. In each case the virus was killed while the controls were fully virulent. No difference in the nature of the two could be detected by this means.

Effect of heat.—In regard to the thermal death point of the virus in rabid brain emulsions, Marie[1] states that the length of time required

[1]Loc. cit.

to weaken and finally destroy the virus by heat varies according to the thickness of the emulsion, or to express it otherwise, according to the number of specific germs present.

It was not possible to standardize the emulsions for comparison, but our experiments showed no marked difference between the fixed brain virus and gland street virus, although the original gland virus was the weaker as shown by the longer incubation when inoculated into guinea-pigs.

Exposure for 10-15 minutes to 48-50° C. weakened both viruses, while the same temperature for 30 minutes killed the gland virus, and greatly weakened the fixed brain virus, only one guinea-pig out of three inoculated developing rabies and that with a prolonged incubation.

Both viruses were destroyed when kept at 60° C. for 30 minutes as shown by Table 5.

TABLE 5

EFFECT OF HEAT

No. of Test	Material Inoculated	Heated	Guinea-Pigs Inoculated	Individual Incubation	Average Incubation
					Days
1....	Glycerin extract gland virus dialyzed in distilled water for one hour (Control)	.....		12 days 11 " 11 "	11 1-3
2....	Fixed virus brain emulsion dialyzed same as above	.....	3	5 " " 1 dead in 1 day; not rabies	4
3....	Fixed virus bran emulsion	48–50° C. for 15 min.	3	5 days 6 " 6 "	5 2-3
4....	Glycerin extract gland virus dialyzed for 1½ hours in salt solution	48–50° C. for 10 min.	2	1 dead in 60 days 1 dead in 10days; not rabies	60
5....	Fixed virus brain emulsion	48–50° C. for 30 min.	3	9 days; 2 discharged in 62 days	9
6....	Glycerin extract gland virus dialyzed for 1½ hours in salt solution	48–50° C. for 30 min.	3	Discharged in 62 days	.....
7....	Glycerin extract gland virus dialyzed for 1½ hours in salt solution	60° C. for 10 min.	3	Discharged in 48 days	.....
8....	Fixed virus brain emulsion dialyzed same as above	60° C. for 30 min.	3	Discharged in 48 days	
9....	Glycerin extract gland virus dialyzed for 1½ hours in salt solution	60° C. for 30 min.	3	Discharged in 48 days	.....

Effect of Chemicals.—The chemicals tested on the brain, centesimal emulsion of fixed virus, and the aspiration gland virus were bichlorid of mercury, hydrochloric acid, and magnesium dioxid. With none of these was there any marked difference in the resistance of the two viruses. A

1-1000 solution of bichlorid of mercury killed both viruses. A 1-1000 solution of hydrochloric acid killed the brain virus and markedly weakened the gland virus, only one guinea-pig out of three contracting rabies with a prolonged incubation. Magnesium dioxid was tried because of its oxidizing effect. In the strength of one-fourth of a gram to four cubic centimeters of the virus, it produced but little effect.

Effect of Dialysis.—Novy and Knapp[1] have shown that dialysis kills fixed rabies virus apparently through the extraction of certain salts and that the time required depends on certain factors such as the thickness of the emulsion, rate of the flow of water, etc. Our results were similar to theirs, though not identical, probably owing largely to the fact of our using much stronger emulsions. We found, *e. g.,* that the dialysis of two cubic centimeters of either brain or gland virus in 200-300 c.c. of standing water had but little effect after 18 hours, but that dialysis of the gland virus in running water for 18 hours killed it. Brain virus on the other hand, while it was markedly weakened, was not completely killed. Dialysis for 43 hours killed the fixed virus emulsion used by us. There were many more tissue elements as well as organisms in the brain virus emulsion used than in the glycerin gland virus which undoubtedly accounts for the longer time required for killing the former virus.

Effect of Concentration.—Some of the filtrate from a Berkefeld V candle was evaporated in a vacuum over H_2SO_4 to one-fifth its bulk—the operation requiring four hours. The control in a narrow tube sealed with paraffin was placed in the apparatus so that conditions of light and temperature would be the same. Three guinea-pigs were inoculated from the concentrated virus as well as from the control and also the concentrated virus diluted to its original volume. The result was that while the pigs inoculated with the control died with an average incubation of 20 days, those from the concentrated virus developed with an incubation of 32 days, while only one pig from the concentrated virus subsequently diluted, developed after 20 days.

This suggests a harmful concentration of salts or proteins, such as is believed by Harris[2] to cause the reduction of virulence in the Pasteur cords.

Effect of Filtration.—In 1903 Remlinger[3] first showed that fixed virus was filterable. He emulsified the entire brain of a fixed virus rabbit in 400 c.c. of water and filtered the emulsion through a Berkefeld V filter under pressure. Ten rabbits were inoculated with from one-half to one cubic centimeter each of the filtrate. Three of these rabbits or 30 per cent. developed rabies. The following year Marie,[4] using three different Berkefeld candles, was unable to pass the virus through in any instance. In the same year, 1904, however, Bertarelli and Volpino[5] successfully filtered fixed virus through a Berkefeld V filter five times out of seven

[1]Jour. Infect. Dis., 1906, 3, p. 291.
[2]Jour. Infect. Dis., 1912, 10, p. 369.
[3]Ann. de l'Inst. Pasteur, 1903, 17, p. 834.
[4]Loc. cit.
[5]Centralbl. f. Bakteriol., I Orig., 1904, 37, p. 51.

TABLE 6

EFFECT OF DILUTION AND CONCENTRATION

Number of Test	Material Inoculated	Number of Guinea-Pigs Inoculated	Number Infected	Individual Incubation	Average Incubation
				Days	Days
1*a*.....	Control virus aspiration gland No. 1	3	3	9 9 8	8 2-3
1*b*.....	Above virus diluted 1 to 4	3	3	8 9 11	9 1-3
2*a*.....	Aspiration gland virus No. 2	2	2	8 8	8
2*b*.....	Above virus diluted 1 to 8	4	4	12 9 9 9	9 3-4
3*a*.....	Aspiration gland virus No. 3	2	2	10 10	10
3*b*.....	Aspiration gland virus No. 3 diluted 1 to 10	4	3	9 11 17	12 1-2
3*c*.....	Aspiration gland virus No. 3 diluted 1 to 20	4	4	9 11 13 15	12
4*a*.....	Aspiration gland virus No. 5	2	1	8 (1 dead in 2 days; not rabies)	8
4*b*.....	Aspiration gland virus No. 5 diluted 1 to 30	3	3	10 9 12	10 1-3
5*a*.....	Aspiration gland virus No. 6	3	3	16 19 25	20
5*b*.....	Aspiration gland virus No. 6 concentrated over sulfuric acid in vacuum (5.5 c.c. to 1.2 c.c.)	3	3	19 33 45	32 1-3
5*c*.....	Same virus as 5*b*. After concentration diluted to original volume with salt solution	3	1	20	20

trials. They failed to pass it through a Chamberland F. Celli and du Blasi[1] had similar results. In 1904 also Di Vestea[2] made extensive experiments on filtration. He used fixed virus and prepared the emulsions with great care, emulsifying the brains with the aid of quartz sand. He filtered with from two to three atmospheres pressure and inoculated his animals with large amounts of the filtrate, *i. e.*, one-half to one cubic centimeter each. His results were as follows:

[1]Ann. d'ig. sper., 1904, 3, p. 603.
[2]Riv. d'ig., 1905, 16, p. 158; La Med. Ital., 1904, 2, p. 241.

	Experiments	Virulent Filtrates	Per Cent.
Berkefeld V	19	14	75
Berkefeld N	1	1	....
Chamberland F	8	2	25
Chamberland B (Filtrate from Chamberland F)	1	0	....
Maasen Filter (Filtrate from Berkefeld)	1	1	....

The literature contains very little regarding the filtration of gland virus. In one instance a virulent filtrate was obtained by Bertarelli and Volpino, who passed it through three layers of paper. It is also recorded that the virus in human saliva was passed in one instance through a Berkefeld candle.

Our experiments were made with Berkefeld V and N, Chamberland F and B, and with a collodion filter. The aspiration virus from the glands of from two to four dogs were used in each experiment. The results were as follows:

	Experiments	Virulent Filtrates	Per Cent.
Berkefeld V	4	4	100
Berkefeld N	1	1	....
Chamberland F	4	3	75
Chamberland B	2	0	....
Collodion Sac	2	0	....

In the collodion sac and the first Chamberland B experiment, the virus unfortunately proved to be quite weak. In the second Chamberland B experiment, however, a strong virus was obtained, but inoculations with the filtrate failed to show symptoms of rabies after a lapse of six weeks.

In order to get some idea of the amount of virus held up by the filter, a series of dilutions of the fluid to be filtered was made as follows: Dilutions of 1 to 4, 1 to 8, 1 to 10, 1 to 20, and 1 to 30. It was found that the average incubation of the guinea-pigs inoculated was increased in a surprisingly regular manner, according to the degree of the dilution of the virus. For example, the dilution of 1 to 4 gave an incubation of nine and one-third days, as against eight and two-thirds days for the undiluted virus, an increase of two-thirds of a day only. A dilution of 1 to 30 gave an incubation of ten and one-third days as compared with eight days for the original virus, an increase of two and one-third days (see Table 6). The average duration of incubation, then, may be taken as a rough index of the dilution of virus. All of the guinea-pigs inoculated with the Berkefeld filtrates developed rabies. That more of the virus passes through after saturation of the filter is seen from the following experiment. A small Berkefeld V candle was used. The first five cubic centimeters which passed constituted the first filtrate, the next 12 c.c., the second filtrate. The four guinea-pigs inoculated with the first filtrate developed rabies with an average incubation period of 21 days. Those with the unfiltered material diluted eight times developed in nine and one-half days and the undiluted control in eight days. Comparing the incubation of the pigs injected with the second filtrate, twelve and

one-half days, with that of the pigs injected with the control diluted 1 to 8, nine and one-half days, it is fair to assume that in this case the virus was diluted by filtration more than eight times. Again in the finer Berkefeld, Grade N, the average incubation of the pigs injected with the second filtrate was fifteen and one-half days, and that produced by the virus diluted 1 to 20 was only eleven and one-half days. In a further attempt to determine the dilution caused by filtration, a Berkefeld V candle was shaved to about one-half its thickness. In this case a weak virus diluted 1 to 20 gave an incubation of 17 days while the filtrate from the shaved filter gave 16 days, practically the same, so that it may be assumed that the shaved filter diluted the virus about 20 times. The filtrates from the Chamberland filters gave correspondingly increased incubation. Thus in one test, two out of four pigs inoculated developed rabies with an average incubation of 33 days, as against eight and one-third days for the control. In a second test two out of five pigs developed with an average incubation of 47 days as against 11 days for the control. The filters were tested with a suspension of pyocyaneus bacilli or other bacteria which was mixed with the virus to be filtered. In no case did the bacilli pass through.

TABLE 7

FILTRATION THROUGH BERKEFELD V FILTER

No. of Test	Materia Inoculated	Number of Guinea-Pigs	Number Infected	Individual Incubation	Average Incubation
				Days	Days
1....	Glycerin extract gland virus (Control) dialyzed	5	2	12 18	15
2....	First 5 c.c. of filtrate of Test 1	5	4	..	Discharged 3 months later, not rabid
3....	Last (30 to 35 c.c.) of the filtrate of Test 1	5	1	56	56
4*a*...	Aspiration gland virus (4 sets of glands) control diluted 1 to 8	4	4	12 9 9 8	9½
4*b*...	Virus of Test 4 undiluted	2	2	8 8	8
5....	Virus of Test 4 passed through filter (first 5 c.c. of filtrate)	4	4	18 26 14 26	21
6....	Last 12 c.c. of the filtrate of Test 4	5	4	12 14 13 11	12½
7....	Aspiration gland virus (3 sets of glands) passed through the filter	3	3	16 19 25	20
8....	Virus of Test 7 kept in icebox 6 days, showed evidence of bacterial contamination, passed through filter	3	2	1 dead in 3 days; not rabies 14 15	14½

Tests 1, *2 and* 3.—The glycerin extract of two sets of rabid glands was dialyzed in one and one-half hours and then passed through a new Berkefeld V filter with the aid of a vacuum pump. The first five cubic centimeters, called the first filtrate, were inoculated in guinea-pigs; the next 25 c.c. were discarded (used to coat or saturate the filter) and the last five cubic centimeters were called the last filtrate and inoculated into guinea-pigs. The original virus was very weak, only two out of five guinea-pigs contracting rabies in Control Experiment 1. The last filtrate was virulent but not the first.

Tests 4*a*, 4*b*, 5*and* 6.—Aspiration gland virus of four sets of rabid glands, amounting to 50 c.c. (much hemoglobin and very cloudy), was passed through a new Berkefeld V filter with the aid of a vacuum pump. The first five c.c., called the first filtrate, were inoculated into guinea-pigs; the next 12 c.c., called the last filtrate, were also inoculated. The filter was then blocked. The last filtrate proved more virulent than the first.

Tests 7 *and* 8.—Aspiration gland virus of three sets of rabid glands which were kept in the icebox four to six days was passed through a new Berkefeld V filter[1] by the aid of a vacuum pump. The first 15 c.c. were used to saturate the filter and the following seven cubic centimeters used for the experiment in Test 7. In Test 8 part of the original unfiltered virus used in Test 7 which had remained in the icebox for six days and showed evidence of bacterial contamination was passed through a new Berkefeld V filter with the aid of a vacuum pump. Eight cubic centimeters were used to saturate filter and the following six cubic centimeters clear but of a reddish color and offensive odor, were injected into guinea-pigs, and proved virulent.

Comparing our results with the filtration of gland virus, with those obtained by others in the filtration of the fixed brain virus, one may infer that the greater regularity with which the gland virus passes (that is, 100 per cent. in the Berkefeld as against 75 per cent., and 75 per cent. in the Chamberland F as against 25 per cent. obtained by Di Vestea) is due to

TABLE 8

FILTRATION THROUGH BERKEFELD N FILTER

Number of Test	Material Inoculated	Number of Guinea-Pigs	Number Infected	Individual Incubation	Average Incubation
				Days	Days
1.....	Aspiration gland virus (2 sets of glands; control diluted 1 to 20	4	4	13 14 10 9	11½
2.....	Virus of Test 1 filtered through Berkefeld N	4	4	14 16 16 16	15½

[1]New filters, generally the small size, were used in all filtrate experiments. The filter was washed in distilled water, then boiled in 0.4 per cent. salt solution for 20 minutes. Before filtration was started the filter was partially drained by the aid of the vacuum. All filters were tested with a suspension of bacteria, usually pyocyaneus or diphtheria and proven perfect.

the freer condition of the virus in the glands. That the organism is not necessarily smaller in this location is seen from the fact that the fixed virus did pass the Chamberland filter, and even in one instance, according to Di Vestea, the Maasen filter.

Aspiration gland virus of two sets of rabid glands was passed through a new Berkefeld N filter with the aid of a vacuum pump. The first 15 c.c. were used to saturate filter. The next 10 c.c. were tested in Test 2 and proved virulent.

A culture of *B. alvei* was used for the test of the filter, and the filter proved bacteria proof.

TABLE 9

FILTRATION THROUGH PASTEUR CHAMBERLAND F

No. of Test	Material Inoculated	Number of Guinea-Pigs	Number Infected	Individual Incubation	Average Incubation
				Days	Days
1...	Aspiration gland virus (3 sets glands.) Control	5	4	8 10 9 12 1 dead in 2 days, not rabies	9 3-4
2...	Virus of Test 1 passed through filter	5	1	26	26 (4 pigs discharged 4 months later, not rabid)
3...	Aspiration gland virus (4 sets glands). Control	2	1	11 1 pig disappeared	11
4...	Virus of Test 3, first 12 c.c. filtrate, passed through small filter	5	2	46 48	47
5...	Virus of Test 3, first 20 c.c. of filtrate, passed through large filter	6	.....	1 dead in 10 days, not rabies *1 dead in 17 days	Other pigs discharged 4 months later, not rabid
6...	Aspiration gland virus (2 sets glands). Control	3	3	9 8 8	8 1-3
7...	Virus of Test 6 passed through large filter and first filtrate used	4	2	27 39	33
8...	Virus of Test 6 passed through large filter and last filtrate used	5	.....	1 dead in 5 days 1 dead in 40 days No Negri bodies seen	Other pigs discharged 4 months later, not rabid

* Guinea-pigs (4) inoculated from this brain died in two days. The brain was then put into glycerin and three days later three more pigs were inoculated from this material. Two died on the next day and the other was discharged four months later, not rabid.

Tests 1 and 2.—Aspiration gland virus of three sets of rabid glands passed through a new Chamberland F filter. Fifty cubic centimeters of the extract were put through the filter of which 35 c.c. were recovered and used in Test 2. Pyocyaneus broth culture used for efficiency of filter, filtrate sterile.

Tests 3, 4 *and* 5.—Aspiration gland virus of four sets of rabid glands was divided and passed through two new Chamberland F filters, one small size and one large. Filtration with the aid of a vacuum pump. In Test 4, 12 c.c. were passed through small filter of which four cubic centimeters were recovered. In Test 5, 20 c.c. were passed through large filter and seven cubic centimeters recovered. Pyocyaneus broth culture used for efficiency of filter, filtrate sterile.

Tests 6, 7 *and* 8.—Aspiration gland virus of two sets of rabid glands was passed through a new Chamberland F filter, large size, with the aid of a vacuum pump. The filtration was slow. The first five cubic centimeters to pass through, called first filtrate, were used in Test 7 and proved virulent. The next 15 c.c. were used to saturate filter and discarded. The last five cubic centimeters, constituting the last filtrate, were tested for virulence in Test 8 and proved non-virulent. A broth culture of diphtheria was used for the efficiency of the filter. Filtrate sterile.

TABLE 10

PASTEUR CHAMBERLAND B FILTRATION

No. of Test	Material Inoculated	Number of Guinea-Pigs	Number Infected	Individual Incubation	Average Incubation
1...	Aspiration gland virus (5 glands from 3 dogs). Control	2	1	1 dead, not rabies 7 days	7 days
...	Virus of Test 1 filtered through large Pasteur B filter	5	.....	1 dead, 3 days 1 " 104 " 1 " 107 " } Not rabies	Other pigs discharged 4 months later, not rabid
3...	Aspiration gland virus (Control) 1–20 dil.	3	3	12 days 12 " 17 "	13 2-3 days
4...	Virus of Test 3 filtered through large Pasteur B filter	5	.....	.....	Discharged 4 months later, not rabid
5...	Virus of Test 1*a*, Table 11, filtered through small Pasteur B filter	5	.....	.....	Discharged 4 months later, not rabid

Tests 1 *and* 2.—Aspiration gland virus of five rabid glands from three dogs was passed through a new Pasteur B filter with the aid of a vacuum pump. Before filtration, 25 c.c. of the virus was mixed with one cubic centimeter of a broth suspension of pyocyaneus and proved filter bacteria proof. The filtrate proved non-virulent.

Tests 3 *and* 4.—Aspiration gland virus diluted one to twenty in salt solution to which two loopfuls of a moist agar culture or pyocyaneus had been added was passed through a new large Pasteur B filter with the aid of a vacuum pump. Seventy-five cubic centimeters were filtered through in 15 minutes, then 25 c.c. of undiluated virus were further filtered through. As the filter had not been completely drained in the beginning 115-120 c.c. were recovered, and, inoculated in the guinea-pigs, proved non-virulent.

Test 5.—Aspiration gland virus was filtered through a shaven Berkefeld V filter (used in Test 1*a*, Table 11). This filtrate was shown to be virulent. It was refiltered through a new Pasteur B filter, small size; about 12 c.c. were passed through of which six cubic centimeters were recovered and proved non-virulent.

TABLE 11

FILTRATION THROUGH COLLODION SAC

Number of Test	Material Inoculated	Number of Guinea-Pigs	Number Infected	Individual Incubation	Average Incubation
1*a*.....	Aspiration gland virus No. 1*a* filtered through shaven Berkefeld V filter. Control	3	2	17 days 17 "	17 days
1*b*.....	Aspiration gland virus No. 1*a* filtered through a collodion sac	5	0	.....	Discharged 3 months later; not rabid
1*c*.....	Aspiration gland virus No. 1, unfiltered, diluted 1 to 20. Control	3	3	12 days 12 " 17 "	13 2-3 days
1*d*.....	Same virus as No. 1*c* filtered through a collodion sac	5	0	.....	Discharged 3 months later; not rabid

Tests 1*a and* 1*b*.—Parts of the aspiration gland virus that was filtered through a shaven Berkefeld V filter was used in Test 1*a*. Then 3.5 c.c. of it were filtered through a collodion sac with the aid of a vacuum of 2.5" to 3". The filtration required two and one-half hours. The filtrate tested in 1*b* proved non-virulent.

Test 1*d*.—Dilution of virus 1 to 20 used in Test 1*c* filtered through under same conditions as 1*b*. Filtration for 3.5 c.c. took five and one-half hours. Filtrate non-virulent. The sacs were tested by pressure before and after filtration and no leakage found.

ATTEMPTS AT CULTIVATION

In the first method tried, the spinal cord of a fixed virus rabbit four days after subdural inoculation was used. At this time the virus has reached the cervical end but has not extended to the lumbar extremity. The cord was quickly removed from the canal by the Oshida method, and blocked into previously sterilized paraffin of 46° melting point, the whole block being then immediately solidified in ice water. In this way a partial anaerobic condition was obtained ,a condition considered by Marie to be desirable. At the end of 10 days not only had the virus not extended to the lower end, but it had died out in the cervical end.

Attempts were made to grow the virus or at least to get an idea of its viability, by making use of the technic of Harrison's method of tissue growing *in vitro*. It was found that apparently the brain cells retained their vitality at incubator temperature for several days, in one instance as long as 21 days. This was inferred from the perfect staining of the Nissl bodies. However, in spite of the ganglion cells remaining alive,

small portions of the fixed virus brains imbedded in plasma, agar, or in simple saline solution, lost their virulence after seven days at 37° C.

On the other hand, in the only instance in which street virus brain was tried, it was found that the virulence was retained after eight days at 37° C.

Portions of normal guinea-pigs brain inoculated with weak emulsion of fixed virus showed no virulence after six days. Normal brain was also inoculated in a similar manner with gland virus, but here also there was no evidence of virus at the end of six days. Unfortunately in this experiment a glycerin extract virus was used which proved to be very weak. From our subsequent work, as well as from clinical observations, we would conclude that the most promising virus to use for this purpose would be the strong aspiration virus freed from bacteria by passage through a Berkefeld filter. This would avoid the objection to brain virus for the cultivation of the rabic organism made by Marie on the ground that at incubator temperature the brain tissue develops substances detrimental to the virus.

Microscopical Examination

The glycerin extract dialized in distilled water or in salt solution, even after concentration through a collodion sac or by evaporation in vacuum, gave a product which, while containing the organism, showed remarkably little in the way of formed elements of any kind. The aspiration virus when filtered through a Berkefeld V candule showed many small granules, of which some were evidently from the secreting cells of the gland, as they were seen also in extracts from the normal gland. Many examinations were made of both of these viruses with the dark field (1–12 and 1–16 lenses and 4 and 8 ocular), also unstained in light field and stained by the various intensive and special methods. In all cases small granules easily seen in the unstained preparations and taking most of the stains with comparative ease were found; but these were found in the extracts of normal glands too, although it occasionally seemed to us that there were more granules in the rabid extracts. Sometimes a ring was seen with a central point and, rarely, slender, slightly curved structures, suggesting cilia, were found. We were unable, however, to identify any of our findings as the specific organism.

Attempts at Agglutination

Anti-rabic serum was produced by immunizing guinea-pigs and rabbits with fixed virus, according to Marie's method. This serum was tested for its agglutinative properties against the rabid gland extracts. Since rabid nerve tissue of the rabbit was used as the antigen, and the rabid gland extract of the dog for the agglutination test, it was hoped we might get a specific agglutination of the organism of rabies, with tissue reactions excluded, a result unobtainable by the use of rabid nerve tissue for both immunization and agglutination.

Preliminary experiments showed in the extracts of the glands of both normal and rabid dogs, an agglutination of granules, apparently greater

in the rabid extracts than in the controls. The results were not sufficiently definite to warrant any conclusions. Further work is being carried on in this line and also with the opsonic technic, and results will be given in a later paper.

III.—Summary and Conclusions

By our two new methods, glycerin extraction and aspiration, we have been able to obtain the virus of rabies from the submaxillary glands, free from the cells of the host and from contaminating organisms. We were thus able to study the virus in a fairly pure state and to compare it with the virus as it exists in the nervous tissue.

Forty-five glycerin extracts of the submaxillary glands of dogs (street rabies) were tested for virulence by subdural inoculations in guinea pigs. Thirty-three or 73.33 per cent. were virulent, the average incubation periods ranging from eight to eighty-two days. A single extraction of glycerin did not always extract all the virus, for in some cases tested, a second glycerin extract of the same glands also proved virulent. An emulsion from glands which had given a non-virulent extract was virulent, showing the action of the glycerin in extracting the virus to be largely mechanical, a sucking out of the virus from the tubules and ducts.

Glycerin extracts of eleven fixed virus brains (rabbit) were virulent in only four instances, while four glycerin extracts of street virus brain (dog) were all non-virulent, showing in all four virulent extracts from fifteen rabid brains—that is, 26.66 per cent. virulence as compared with 73.33 per cent. of the glycerin gland extract. Considering the great virulence of fixed virus brains, one would expect a greater number of virulent glycerin brain extracts. The result, however, need not necessarily argue a difference of form of the virus in this situation, but rather indicates that the virus is here more intimately associated with the nerve cells, while in the glands it is probably largely a drawing out from the ducts. The few virulent brain extracts might have been due to very minute particles of the brain substance, the brain being more friable than the gland, or to the extraction of the virus from the blood and lymphatic vessels and spaces.

A few glycerin extracts made from the suprarenal capsule, sciatic nerve, and pancreas from the dog (street virus) were all non-virulent.

The aspiration virus of the rabid submaxillary glands was always virulent, usually with an incubation period of from eight to nine days.

The resistance of the virus of rabies to glycerin so frequently taken as suggesting a protozoal origin has been found by us to be paralleled by diphtheria and tubercle bacilli. Cultures of these bacteria, which were subjected to glycerin in the cold and dark, diphtheria bacilli for two weeks, tubercle for thirteen months, on dialysis and transplantation, gave vigorous growths. Their staining properties were not impaired, and the tubercle bacilli were fully virulent; the diphtheria culture was not tested for virulence. As the action of glycerin is largely that of the extraction of water or drying and both the cultures withstand drying though neither has a spore stage, the results are not surprising.

We find, therefore, that these bacteria are not destroyed by glycerin, the tubercle bacillus having a resistance as great as the virus of rabies, which resistance cannot therefore be considered as necessarily indicating a protozoal virus.

In comparing the virus as it exists in the brain and in the gland, this fact must be borne in mind; with viruses which cannot be standardized slight differences in reactions to either physical or chemical agents are not to be interpreted as indicating a difference in their physical or biological state, as variations in the number of organisms present in the concentration of proteins and salts vary the results with the reactions of the different emulsions.

Comparative experiments on the brain and gland virus for viability, for the effect of rapid drying, heat, combined drying and heat, filtration, dialysis, action of glycerin, of magnesium dioxid, of bichlorid of mercury and of hydrochloric acid, have shown no sufficient difference to indicate either a protozoan cycle or a spore bacterial form in the virus of rabies in these two situations.

In our filtration experiments with the gland virus we obtained 100 per cent. virulent filtrates with the Berkefeld filters and 75 per cent. with the Pasteur Chamberland F filter. Di Vestea, working with brain emulsion, secured only 75 per cent. positive filtrates from the Berkefeld filter and 25 cent. from the Pasteur Chamberland F. This difference in results need not argue a smaller form of the organism in the gland. It may be due to the virus being less intimately associated with the cells of the host in this situation, as was previously suggested by the fact of glycerin extract of rabid brains being virulent in only 26.66 per cent. as compared with the 73.33 per cent. of virulence of the glycerin gland extracts.

Whether this would indicate that the chromatin elements of the Negri body may be the true rabic parasite, the outer portion being a degeneration product of the nerve cell, we are at present unprepared to say. The following points are to be considered in this connection. First, the structure known as the complete Negri body could not pass through the filter; second, some of the minute, inner cell-structures might pass; third, the small central structures might easily exist free in the gland and escape identification. In this connection, it is interesting to note that working in conjunction with Dr. R. A. Lambert,[1] we have been able to produce inclusions in ganglion cells, closely resembling the outer portion of Negri bodies, by allowing normal nerve cells to slowly degenerate in plasma at 37° C. according to Harrison's technic for the growing tissues *in vitro*.

Many factors also are to be considered in judging the results of filtration. The question of the coating or saturation of filter is sometimes of great importance, even for the passage of proteins in solution as shown by one of us in complement filtration, and as our present work has shown in the Berkefeld filtrate. Therefore, in any question of filtration the amount filtered, the first or last filtrate tested, the dilution of the organism and of the foreign protein and the amount of salts in the fluid to be

[1] *Jour. Infect. Dis.*, 1912. II, p. 459.

filtered, the pressure used, the possible concentration of the filtrate by evaporation, centrifugation, precipitation, or possible agglutination, all are important factors toward the end results. So the comparison of filtration experiments unless done under identical conditions cannot be taken as absolute proof of difference in size.

The fact of a virus passing through even the Chamberland Pasteur F filter does not mean that it is invisible with our present powers of magnification. The organism of peripneumonia, the classic example of a filtrable virus, readily passes through this filter but is easily seen by the use of the dark field and oil immersion.

Our attempts at cultivating the virus of rabies even with the *in vitro* method have not as yet been successful.

From our microscopic examinations we conclude that the virus of rabies as it exists in the submaxillary gland, although possibly visible, is probably of no distinctive form. By the use of various staining methods, by frequent examinations of many viruses with both light and dark fields, with high magnification, no distinctive formed elements were seen. In all cases small granules were readily found. Occasionally there were rings with a central point and cilia-like structures, but these were also found in the control extracts of normal submaxillary glands. Occasionally it seemed to us that there were more granules in the rabid than in the normal extracts, but no conclusions could be drawn. Experiments on the possibility of obtaining a specific agglutination of the virus of rabies as it occurs in the gland extract from an antirabic serum, obtained by the injection of rabid brain, were tried. By this method it was hoped to exclude tissue reactions. In some cases it seemed as if there were greater clumping of granules in the rabid extracts than in the control normal extracts. Further work is now being carried on along this line and with the opsonic technic with the possibility of a specific phagocytic action locating the virus in the leukocytes. Until these experiments are completed no definite conclusions can be drawn concerning the granules.

In closing, we wish to emphasize the following points:

1. The advantage of using the gland secretions in the study of rabies because they contain the organism freed from tissue cells. This is an advantage for the microscopical study of the virus, for the study of its physical properties, and for its use in growing experiments.

2. The two easy methods for obtaining bacterially sterile virus from the glands.

3. The resistance of bacteria to glycerin which is as great for the tubercle bacillus as for the virus of rabies.

4. The regularity with which the gland virus passes the Berkefeld filter and the large percentage of virulent filtrates with the Chamberland Pasteur F filter.

5. The similarity in the nature of the rabies virus in the two locations of the brain and the glands as shown by the comparative effect of physical and chemical agents.

In conclusion, we wish to express our thanks to Dr. C. Zell, Frederick Jelinek, and Miss L. Alper, who assisted largely in the technical part of the work.

THE PRODUCTION *IN VITRO* IN THE NORMAL BRAIN OF STRUCTURES SIMULATING CERTAIN FORMS OF NEGRI BODIES*

EDNA STEINHARDT, DANIEL W. POOR AND ROBERT A. LAMBERT

The Negri bodies have been interpreted in two ways, by some observers as protozoa, by the majority, however, as degeneration products of nerve cells. In each of these interpretations the bodies are regarded as specific for rabies. Thus far, neither view has been supported by experimental evidence. The following work was therefore undertaken in the hope that some light might be thrown upon the nature of the bodies in question. Although the results are not entirely conclusive, they lend weight to the view of the degenerative origin of at least some of the smaller forms of Negri bodies.

EXPERIMENTAL WORK

The results of the present investigation, which refer, in brief, to certain morphological changes in brain cells under special conditions of incubation, may be conveniently described in three parts, as they relate (1) to the normal brain, (2) to the normal brain inoculated with rabies virus, after the death of the animal, and (3) to the rabid brain.

Technic.—The technic employed consisted simply in an application to our purposes of Harrison's method of cultivating tissues *in vitro*. Small pieces of the brain of rabbits and guinea-pigs were suspended in hanging drops of blood plasma obtained from the animal from which the brain was to be removed. All portions of the brain were used, but Ammon's horn gave the most satisfactory results in that the ganglion cells in this region developed the most numerous degenerative structures. The culture preparations were incubated at 37.5° C. for varying lengths of time. In each experiment a number of preparations (20 to 50) were made in order to allow a serial study of the stained cells. An examination was made every two days after incubation was begun, four to 10 slides being used each time. Van Gieson's[1] method for the rapid diagnosis of rabies, as modified by Williams,[2] was used for staining the specimens.

In this connection we wish to record our indebtedness to Dr. Van Gieson for his generous assistance in this study.

I.—INCUBATION OF NORMAL BRAIN

At the beginning of the present study it was decided to attempt first to produce Negri bodies *in vitro* by combining outside the body rabies virus and living brain cells. This accomplished, it seemed to us that a study of the character of the bodies could more easily be undertaken. With this end in view an experiment with virus-injected guinea-pig brain, as described in the next division of this paper, was made, a control,

*Study made in conjunction with the Department of Pathology, Columbia University, New York City.

[1]Proc. N. Y. Path. Soc., 1906, 5, p. 83.

[2]Ibid., 1905, 5, p. 155.

however, being made in which an uninjected normal guinea-pig brain was used.

In examining smears from the bits of tissue in the hanging-drop preparations after incubation we were interested to find in the ganglion cells in *both* series structures practically identical in appearance with certain forms of Negri bodies found in the rabid brain. To exclude the possibility that some error in technic may have been responsible for the findings in the normal control specimens, further experiments were made using pieces of normal brain only. The results of this study will now be briefly described.

Smears made from the pieces of tissue after four to six days' incubation show ganglion cells with well preserved nuclei. The cells are considerably enlarged. The increase in size involves both nucleus and cytoplasm. The nuclei stain lightly by Van Gieson's method. The cytoplasm shows numerous round clear spaces which represent fat droplets in the living cell. It should be noted here that a similar intracellular accumulation of fat has been regularly observed in the cultivation *in vitro* of the various body tissues. It probably indicates a condition of disturbed metabolism resulting from the changed conditions of life.

In the cytoplasm of a certain number of the cells there are found small pink-staining bodies. These bodies, which we have called "degenerations," are not distinguishable by the usual methods of staining from the small intracellular unstructured Negri bodies observed with great frequency in the rabid guinea-pig brain. Some of the "degenerations" are surrounded by a blue granular ring, such as is seen in typical Negri bodies. In a few instances, structured forms were also found showing a small blue-staining ring in the center. These "degenerations" closely resemble the small structured forms of Negri bodies in the rabid brain.

In addition to the two intracellular forms, extracellular unstructured forms were also found. Specimens incubated for a longer time (10 to 20 days) did not show any further changes in the character of the structures.

In one experiment in which the brain of a normal puppy was used, the results were unsatisfactory owing to the rapid degeneration of the ganglion cells in the cultures. Whether this was due to faulty technic or to exceptional delicacy of the brain cells of the dog, it will be necessary for further work to determine. Since the Negri bodies found in the brain of a rabid dog have a more complex structure than those in the guinea-pig, the dog's brain should be better suited to studies such as we have undertaken. Further experiments in this direction are contemplated.

II.—Incubation of Brains Inoculated With Virus

Two series of experiments were made using brains from freshly killed animals injected *in situ* with rabies virus. In both series normal guinea-pig brains were used.

Series 1.—Street virus; gland extract: The submaxillary glands of

a dog dying of rabies (street virus) were extracted under sterile conditions with glycerin. The glycerin was removed by dialysis and the virus concentrated in physiological salt solution by the use of collodion sacs. The virus was injected thoroughly into the substance of the brain by means of a hypodermic syringe. Hanging-drop plasma preparations were made, and incubated as in the preceding set of experiments.

Series 2.—Fixed virus; brain emulsion: The brain of a fixed-virus rabbit was used for making the emulsion. A normal guinea-pig brain was injected as in Series 1, and hanging-drop plasma preparations were made.

In both series the results were identical with those obtained with the normal uninoculated brain; that is, the small intracellular unstructured "degenerations," with an occasional structured form, were found after four to six days' incubation.

III.—Incubation of Rabid Brains

Two series of experiments were carried out using street and fixed viruses.

Series I.—Street virus; guinea-pig's brain: Hanging-drop preparations made with tissue from the brains of guinea-pigs dying of street virus were incubated and examined as in preceding experiments.

Stained smears of the brain before incubation showed mostly the small unstructured Negri bodies usually found in the rabid guinea-pig brain. Upon incubation no development of the forms could be observed, and apparently there was no increase in number. It should be noted here that ganglion cells appear to live in the cultures for 10 to 20 days or longer. One preparation, 21 days old, still contained cells that appeared to be living though greatly enlarged. Observed with a 1/12 objective and a No. 4 eye piece, one such cell stretched entirely across the field.

Series 2.—Fixed virus; rabbit brain: Small pieces of the brain of a rabbit dying of fixed virus were incubated and stained as in the preceding experiments. In the incubated material small forms with central structure were somewhat more numerous than in the fresh brain. But since no study has as yet been made of the normal rabbit brain incubated in the same manner, we are unable to say how frequently the unstructured "degenerations," such as were observed in the normal guinea-pig brain, would be found under the same experimental conditions.

IV.—Inoculation of Animals With Incubated Rabid Material

Guinea-pigs were inoculated intracerebrally with the incubated preparations of rabid brains as well as with incubated pieces of normal brains previously injected with virus. The periods of incubation of this material were from six to ten days. There was only one positive result from the inoculations. This was from material from a rabid guinea-pig brain (street virus) incubated for eight days. In this instance, although the virus had lived, there was, in the stained preparations, no evidence that multiplication or development of the Negri bodies had taken place.

Summary

1. The cells of the normal guinea-pig brain, incubated in blood plasma, showed in their cytoplasm, when stained by Van Gieson's method, small pink-staining bodies surrounded by a blue granular ring, indistinguishable from the unstructured Negri bodies observed with great frequency in the rabid guinea-pig brain.

In a few instances these forms contained a central blue-staining ring or point, and closely resembled some of the smaller structured forms of the Negri body.

2. The normal guinea-pig brain inoculated with rabid material, street or fixed virus, incubated in the same manner, showed the same structures.

3. The brains of guinea-pigs dying of street virus, and rabbits dying of fixed virus, incubated in small fragments, gave no development of the Negri bodies in blood plasma, beyond the small structured and unstructured forms, although in one preparation the ganglion cells appeared to be living at the end of 21 days' incubation.

STUDIES ON THE CULTIVATION OF THE VIRUS OF VACCINIA*

Edna Steinhardt, Clara Israeli and Robert A. Lambert

Many investigators have shown that the virus of vaccinia, except in a dried form, retains its virulence but a short time at 37° C. The virus is usually found inactive after 9 to 13 days' incubation, although under special conditions it has been kept as long as 16 to 21 days. No definite subcultures, however, have been obtainable.

We wish to refer in some detail, in this connection, to the work of Williams and Flournoy.[1] They inoculated with vaccine virus the corneas of rabbits, which were removed 24 hours after inoculation and placed in tubes of ascitic broth. The tubes were incubated at 36° C. After 24 hours' incubation the loosened epithelial cells formed an emulsion in the fluid. The inoculation of this emulsion on the skin of a rabbit gave a typical eruption. In one experiment, in which transfers to fresh broth were made on alternate days, the inoculations of the second and third transfers on the cornea of a rabbit produced typical ulcers. Inoculations from the fifth and sixth transfers on the skin of a calf gave a few scattered pustules; inoculations of subsequent transfers were negative. The inoculations of the fourth, fifth and sixth transfers on the skin of rabbits were negative. The authors attribute the activity of the transfers to a carrying over of the original virus, since they saw no evidence of multiplication. The total period of incubation of the virus in their experiments was from 10 to 12 days.

Experimental Work

In previous work with the virus of rabies, done in conjunction with D. W. Poor,[2] we applied Harrison's method of growing tissue *in vitro* to brain tissue and were able to produce inclusions in normal ganglion cells closely resembling certain small forms of Negri bodies, but were not able to cause any multiplication of the virus of rabies. After these results with rabies, we decided to continue the work with Harrison's method, applying it in the present experiments to the virus of vaccinia incubated with corneal tissue and plasma, again, with two objects in view: the possible production of vaccine bodies *in vitro*, and the possible cultivation of the virus of vaccinia outside of the body.

The virus was taken from the stock of glycerinated, carbolized, calf virus as distributed by the Board of Health of New York City. It was dialyzed through collodion sacs by the method of Poor and Steinhardt.[3] It was usually allowed to stand in the icebox over night, thus permitting the coarse particles to settle. The supernatant fluid was drawn off and diluted with Ringer's or physiological salt solution. By this means a fairly uniform emulsion is obtained.

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* Study made in conjunction with the Pathological Laboratory, College of Physicians and Surgeons, New York City.

[1] Studies from the Rockefeller Institute, 1905, 3, p. 146.

[2] Jour. Infect. Dis., 1912, 11, p. 459.

[3] Jour. Infect. Dis., 1913, 12, p. 202.

Technic.—Small pieces of rabbit or guinea-pig cornea were placed for a few minutes in this weak emulsion of virus. The pieces were then transferred with a small quantity of the virus to cover-glasses to which drops of rabbit or guinea-pig blood plasma were added. The cover-glasses were immediately inverted and sealed over hollow-ground slides, which were incubated at 37° C. To control the microscopic work, similar preparations were put up without virus, and to control the necessity of the cornea in the animal inoculation work, pieces of paraffin were substituted for cornea in a series of preparations.

Microscopic Studies.—Approximately 100 hanging-drop preparations upon five series of experiments were studied histologically, using several methods of fixation and staining. Examinations were also made on fresh unstained preparations with light and dark field illumination. The preparations were stained after varying periods of incubation. As a rule, four slides were taken out at once and studied in several ways. To fix a specimen, the cover-glass with the attached clot was dropped directly into a dish of the fixing fluid unless the fixative was bichlorid, when, according to the method of Lambert,[1] it was first soaked for ½ hour in salt or Ringer's solution to prevent the precipitation of proteins giving subsequently a cloudy specimen. The subsequent staining was carried out as though the specimen were a paraffin or celloidin section attached to a slide, except that on account of the thickness of the hanging drop a longer time is required in the process.

Briefly stated, the methods employed were: (1) Zenker fixation, followed by eosin-methylene blue stain; (2) formalin fixation, Sudan III iron hematoxylin stain; (3) Giemsa's stain with and without previous fixation; (4) eosin-methylene blue without previous fixation; (5) unstained preparations with direct and dark field illumination.

The results of these studies may be given in a few words. The corneal epithelium shows an active lateral spreading through the clot, forming sheets or groups of cells in the plasma. The cells early show an accumulation of fat in their cytoplasm, but may retain their form for several weeks even when not transferred to fresh plasma. Careful studies have failed to reveal any specific vaccine bodies in the preparations; only smaller, undifferentiated forms have been seen and these have been found in both the controls without virus and the virus preparations after incubation.

We have also studied the corneas of guinea-pigs, inoculated with virus, which were removed 24 hours later and put up in hanging drops as described above. Only the smaller forms of the vaccine bodies were found in the beginning, and no further developments were seen. These negative findings are of interest on account of the results of the animal inoculations of the incubated preparations as given below.

Although we have observed numerous granules in the incubated preparations, these have not been sufficiently definite in character with the methods employed thus far in our studies to allow us as yet to make any positive statements in regard to them.

[1]Bull. 4, International Association of Medical Museums, 1913.

We wish here to record our indebtedness to Dr. A. W. Williams for her very helpful criticisms and suggestions in the microscopic work just described.

Animal Inoculations

To demonstrate the activity of the virus, we have adopted the method of Calmette and Guerin.[1] They have shown that the virus rubbed on

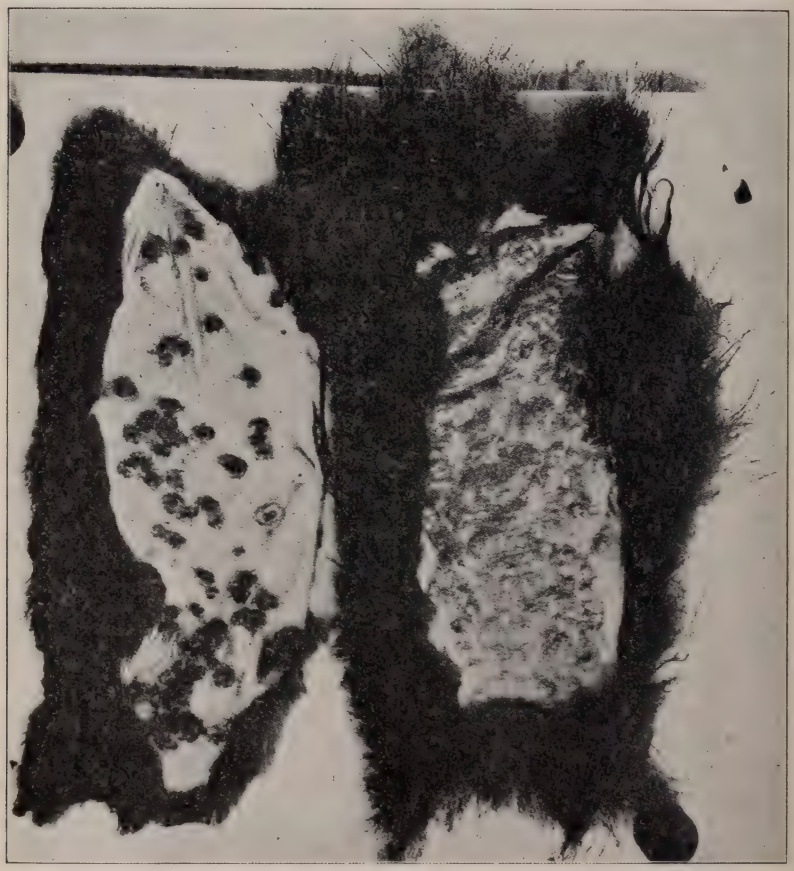


Fig. 1. Fig. 2.

Fig. 1.—Right side of rabbit inoculated with 9 unincubated cornea preparations. Result: 50-55 pustules.

Fig. 2.—Left side of rabbit inoculated with 9 cornea preparations, same series, incubated 14 days. Result: confluent raised eruption.

the freshly shaven skin of the rabbit produces a typical vaccinia eruption, and, furthermore, by methods of dilution, that the number of vesicles in the eruption indicates approximately the quantity of virus present.

[1]Ann. de l'Inst. Pasteur, 1905, 19, p. 317.

This method is generally used in commercial laboratories for standardizing the virus.

In our experiments, the skin of a rabbit was inoculated at once with a small number of *unincubated* preparations, and similar inoculations were made later with *incubated* preparations to determine whether or not there was an increase in the virus.

The "takes" with unincubated preparations, using eight different viruses, varied from 10-50 vesicles, whereas a similar number of preparations incubated 7-18 days gave in every instance an extensive confluent eruption, showing that a definite increase had taken place. Repetitions of these experiments with the different viruses have in all cases given the same results. As yet we have not found a virus that has not shown increased activity under these conditions of incubation. Preparations containing small pieces of paraffin showed, on the other hand, no increase in activity upon incubation.

The following protocols are examples of the results obtained.

Experiment R. 490.—A series of hanging drop plasma preparations were made with rabbit cornea and Virus A. A second series were made using small pieces of paraffin in place of cornea, care being taken to make the quantity of virus the same for both series.

A rabbit inoculated on the skin of the back with 12 unincubated preparations presented on the third to fifth day 9-10 vesicles. No later lesions appeared.

A rabbit inoculated on the left side with 9 cornea preparations incubated 7 days gave an extensive, raised, confluent take. We estimated that at least 200 pustules must have been present to have given a confluent eruption over the area involved (Fig. 4). The same rabbit was inoculated simultaneously on the right side with 9 paraffin preparations also incubated 7 days. Five pustules were observed on the fifth day, and there were no further developments. (See Fig. 3.)

Experiment G. 422.—Hanging-drop specimens were prepared with guinea-pig cornea and Virus B. Nine unincubated preparations used for immediate skin inoculation gave 20-30 lesions. (See Fig. 1.) An inoculation with a similar number of preparations incubated 14 days gave an extensive confluent take. (See Fig. 2.)

Experiment G.—Hanging-drop plasma preparations were made using guinea-pig cornea and Virus C and were incubated at 37° C. On the ninth day of incubation nine preparations were used for a rabbit skin vaccination. A typical rash appeared before the fifth day, in which 40-50 pustules were counted and on the seventh day, 55 pustules. Nine preparations incubated 18 days were used for vaccinating a second rabbit. An extensive, elevated, confluent rash resulted composed of at least 200-250 lesions. (See Fig. 2.)

Subcultures of the preparations have been made and successful skin inoculations have been obtained from the third transfer. The virus has remained active after 34 days' incubation, and this obviously does not represent the limit of activity under such cultural conditions. In control

experiments it was found that the virus alone was greatly weakened after a week's incubation, and that after three weeks' incubation, inoculations on rabbits were negative although much larger quantities of the



FIG. 3. FIG. 4.

FIG. 3.—Right side of rabbit inoculated with 9 paraffin preparations incubated 7 days. Result: 5 pustules (other marks are crusts from shaving wounds).

FIG. 4.—Left side of rabbit inoculated with 9 cornea preparations incubated 7 days (same series as paraffin preparations). Result: confluent raised eruption. (Inoculation with 12 similar cornea preparations, same series, unincubated, was followed by a take of 10 pustules.)

virus were used than with the tissue preparations. Experiments undertaken with the object of obtaining a growth of the virus in test tubes have thus far given interesting and encouraging results.

Work on this point, with further experiments on subcultures, on the use of other organs and the necessity of living tissue in the culture experiments, methods of obtaining a sterile virus, immunity reactions *in vitro,* and the results of further microscopic studies will be described shortly in a later communication.

Conclusion

From our work thus far on the application of Harrison's method to the cultivation of tissue *in vitro* to corneal tissue plus the virus of vaccinia, we are able to state that there is a multiplication of the virus of vaccinia although no specific vaccine bodies are found in the preparations.

STUDIES ON THE CULTIVATION OF THE VIRUS OF VACCINIA*

SECOND PAPER

EDNA STEINHARDT AND ROBERT A. LAMBERT

Two years ago[1] we began to apply the method of cultivating tissues in vitro (Harrison) to the study of the viruses of certain infections in which the specific living agents have not yet been identified. The two viruses studied thus far with the new method have been those of rabies and vaccinia. In the study of the former, we attempted, first, to determine whether the Negri bodies, the nature of which is still in dispute, would show any development or multiplication *in vitro* when fragments of brain from rabid animals were incubated in blood plasma, and, secondly, to see if the characteristic bodies could be produced *in vitro* by combining normal brain and virus. We found that no development or multiplication took place *in vitro,* and that, although structures indistinguishable from certain forms of Negri bodies developed in the virus-normal brain preparations, the same bodies were found in control preparations, in which were pieces normal brain without virus. In a single instance the virus remained alive as long as eight days of incubation; animal inoculations of preparations incubated for a longer time gave in all cases negative results.

During the past year we have been applying Harrison's method to the cultivation and study of the virus of vaccinia. The results of our first series of studies appeared recently[2]. We concluded that there was a definite multiplication of the virus in tissue cultures composed of blood, plasma and pieces of cornea (of rabbit or guinea-pig) to which small quantities of diluted virus were added. Successful animal inoculations were obtained from the third subculture. Histological studies of the preparations, however, revealed no specific vaccine bodies. The present paper is concerned with the continuation of these experiments.

Levaditi[3] has recently applied Harrison's method with some success to the cultivation of the virus of poliomyelitis.

We wish to record at this time experiments upon the following points: (1) Estimation of the extent of multiplication of the virus in cultures by use of higher dilutions of original virus; (2) necessity of *living* corneal tissues for the growth of the virus; (3) possibility of substituting other organ tissues for cornea; (4) use of plasma and cornea from immune animals in the culture preparation.

USE OF HIGHER DILUTIONS OF VIRUS

In previous experiments, described in our first paper[2], the virus was diluted 1:5 before use in the cultures. Inoculation of a small number of unincubated preparations in the skin of rabbits gave in some instances 50-60 pustules; the incubated preparation, on the other hand, produced

* Study made in conjunction with the Department of Pathology, College of Physicians and Surgeons, New York City.

such extensive confluent eruptions that the number of pustules could not be estimated with accurcay. In order to determine the extent of multiplication of the virus, it therefore seemed desirable to make use of a higher dilution of the virus. With this end in view the following experiments were carried out:

Exper. 501. Two commercial glycerinated viruses (2190 and 2193) were dialyzed in salt solution according to the method of Poor and Steinhardt[4] and placed in ice box to allow coarse particles to sediment. Only the supernatant fluid was used.

Virus 2190 was diluted 1:15 in Ringer's solution. A large number of preparations were made with rabbit cornea and plasma, using the same technique as described in our first paper. Nine unincubated preparations inoculated in the skin of a rabbit gave ten flat pustules (sixth day). It should be mentioned that a very dilute virus gives frequently flat pustules with our preparations. The same number of slides incubated eleven days gave upon inoculation 60-65 elevated pustules. Practically all of the preparations in this series showed contamination with bacteria, and, consequently only a slight and transient growth of corneal epithelium. Whether or not bacteria interfere with the growth of the virus in tissue cultures, we are not yet prepared to say, since contamination have been exceptional in our experiments. In this instance the virus was probably the source of the infection.

Virus 2193 was diluted 1:15 in Ringer's solution and preparations made as before. Nine incubated slides inoculated in the skin of a rabbit gave six flat pustules. Inoculation of the same number of preparations incubated sixteen days (contaminated) produced fifty-eight well developed pustules.

These two experiments show, then, that, in spite of the bacterial contamination, which may conceivably interfere with the growth of the specific micro-organism, the virus was increased six to ten fold by incubation in the cornea-plasma preparations. In several other experiments in which a lower dilution of the virus was used, the extent of growth appeared to be greater, but in no instance has the growth been comparable to that observed in cultures of rapidly growing bacteria. Irregularities in the rate of growth are to be expected, since they occur in the cultivation of practically all of the known micro-organisms.

The method used here for estimating the degree of multiplication has been shown by Calmette and Guerin[5] to be reasonably accurate comparing favorably with the method of plating for bacteria; it is in general use in commercial laboratories.

Necessity of Living Tissue for Growth of Virus

We have shown (first paper) that when pieces of paraffin are substituted for cornea in plasma preparations, no growth of the virus takes place. To determine whether or not *living* cornea was necessary, the following experiments were carried out:

Exper. 503. Virus 2186. Diluted 1:5 in Ringer's solution. The pieces of cornea to be used were divided into two lots; those in the first lot

were frozen in an ice-salt mixture at 18° C for thirty minutes, and were then used for virus cultures. The unfrozen tissue was used in control preparations. The frozen tissue upon incubation showed no out growth of either epithelium or connective tissue cells. Nine of the preparations incubated twelve days gave upon inoculation only 12-15 pustules; the same number of control preparations gave a confluent "take" composed of at least three hundred pustules.

Exper. 504. Virus 2186. Diluted 1:5. Pieces of cornea were placed for 45 minutes in weak hypotonic salt solution before using. Inoculation of nine unincubated preparations gave 50-60 pustules. Nine slides incubated ten days were negative upon inoculation. There was also no "take" following the inoculation of five slides incubated twenty-one days. Microscopic examination showed that there was no growth of corneal tissue in any of the preparations. A second experiment was done at the same time using cornea killed by freezing, as in experiment 503. The results were practically identical with those just recorded for cornea killed by hypotonic salt solution.

These three experiments indicate that the vaccine virus does not grow in preparations containing corneal tissue killed by freezing or by a weak hypotonic salt solution. These two methods of destroying the vitality of the tissue were used because the chemical changes which they bring about are very slight. The results obtained are in harmony with the general conception of the close association of the virus with living body cells.

Substitution of Other Tissues for Cornea

Pieces of heart, liver and kidney were used. Since the technique employed was the same as with cornea, it seems unnecessary to describe the experiments in detail. The results were about as follows: The virus died out quickly in the liver preparations. This was due no doubt to the action of bile, which has been shown to be highly toxic for the virus. There was no evidence of multiplication in the heart and kidney preparations; on the contrary, there seemed to be a gradual weakening of the virus. Preparations incubated for more than ten days were found to be practically inactive.

Use of Plasma and Cornea for Immune Animals

Immunity was established in a rabbit by a cutaneous inoculation of virus, resulting in an extensive eruption. Two weeks later the animal was bled, and cornea removed for use in the following experiment.

Exper. 486. Virus diluted 1:5. Two series of preparations were made; one, with plasma and cornea from immune animal; the other, with plasma and cornea from a normal rabbit (controls). Inoculation of five "immune" preparations, incubated five days gave a completely negative result (skin of rabbit, left side). Inoculation of same rabbit simultaneously on right side with an equal number of control preparations, yielded a very extensive confluent "take." Repetition of this experiment a week later gave a similar result. In both experiments there were excellent growths of corneal epithelium.

This experiment shows that the virus becomes inactive in a short time in preparations containing plasma and cornea from an immune animal. The experiment also affords a good demonstration of the adaptability of the method of tissue cultivation for the demonstration of many immunity reactions in vitro.

Conclusions

1. The virus of vaccinia incubated in tissue cultures composed of plasma and cornea from normal rabbits and guinea-pigs shows a definite increase, but the degree of multiplication is not comparable to that observed in cultures of rapidly growing bacteria.
2. There is no growth of the virus in preparation containing cornea killed by freezing or by hypotonic salt solution.
3. There is no evidence of the growth of the virus in preparations in which pieces of heart, liver or kidney have been substituted for cornea.
4. The virus is soon rendered inactive in preparations containing plasma and cornea obtained from an immune animal. The method of tissue cultivation is well adapted for the demonstration of immunity reactions in vitro.

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LIME ASSIMILATION AND TUBERCULOSIS*

Ira Van Gieson

Lime starvation in tuberculosis has been suspected and the hint acted upon often, in a casual way, for a hundred years past. Nothing ever came of it in practice, nor are we any better off in theory.

Even to this day the notion still prevails that lime starvation is so crude and simple a transaction that all we have to do in its correction and counteraction is to prescribe calcium salts and give lime as a drug. But it does not work. In a previous article,† both by reasoning and experiment, I tried to show that calcium medication in the present state of synthetic organic chemistry is no equivalent or substitute for lime assimilation, except perhaps in a feeble and insignificant degree. This statement refers more particularly to calcium medication in experimentally tuberculous guinea pigs and rabbits, and lime assimilation experiments in tuberculous dogs.

By the explanation of lime assimilation is meant a plotting out of the sequence of events therein; its rationale and its *modus operandi*. This gives the key to lime starvation; discloses a new and unrecognized range of phenomena; puts the fields of lime starvation and their attendant or sequential ills under human control and management, and bequeaths a new and extensive domain of practical application and therapy treatment and preventive medicine.

The first year of this examination was spent in Dr. Russell's clinics, watching the effect of lime assimilation in the treatment of pulmonary tuberculosis carried out systematically and persistently. I saw many of these ambulatory patients improve, arrest the tuberculous process, and to all intents and purposes get well. And there were too many of them to think that they would all have gotten well if left to their own devices.

I am sure that measures to promote lime assimilation in tuberculosis can do no possible damage. These measures are not onerous, either for patient or physician or in prophylaxis. On the contrary, they are simple, and I am equally impressed with the good and betterment that can be done by this consideration of tuberculosis, both in the management of the disease, and, greatest of all, in its prevention. In terms of lime starvation and assimilation certain things are conspiring to aid the work of the bacillus, and, fortunately, also certain things in our present civilization, most of all in food supply, are operating to check and offset it.

But we have strayed away from the matter in hand. This was the propriety of subjecting these ideas and possibly restraining some of these contrivances for the remedy or control of tuberculosis, with the verdict

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* This article contains the Laboratory findings with but brief theoretical discussion. A fuller discussion is contained in the report of the proceedings of the Eighth Annual Meeting of the National Association for the Study and Prevention of Tuberculosis, May 30-31, 1912.

† Van Gieson and Lynah, "Creosote and Calcium Medication in Respiratory Affections in Children and in Pulmonary Tuberculosis." Russell's "Generalization in Tuberculosis," New York Medical Record, May 11, 1912.

of rigorous experimental proof. For tuberculosis is one of the few disease processes that can be more or less perfectly reproduced in the laboratory in animals. So if we pretend to control the phenomena of tuberculosis in human beings, it follows that we ought to predict and extend that control on experimentally tuberculous animals.

Observe the advantages of the experimentally tuberculous animals; how nicely the dosage of the bacilli may be measured and adjusted; how well variations in virulence may be introduced; and, finally, how companion animals may be put under precisely the same conditions to check up and counterpoise the remedial experiment. The adaptability and inviting character of experimental conditions as a test for our understanding and reversal of the phenomena of tuberculosis hardly have a parallel in any other disease, and, indeed, in the whole realm of medicine.

So realizing that the badge and token of confidence of any supposed apprehension of the sequence of events in tuberculosis lay in the outcome of the animal test, and that the clinician should not be asked to take such measures on faith when so admirable a set of conditions as artificial tuberculosis are at hand in the laboratory, we planned out the experiment. What was to be undertaken, of course, was the production of artificial and managable lime starvation in animals, and also the restitution of lime nutrition, so as to note the effects of these periods on the course of the infection by virulent tubercle bacilli properly controlled in perfectly normal companion animals.

The problem then was to make the bacillary infection give an account of itself when confronted with fluctuations in lime assimilation, and learn of its response and adaptations to withdrawals and returns of lime nutrition. This sounds simple, easy, but it is extremely tedious and difficult to carry out in actual practice. Dogs seem ideal for this experiment. Here is an animal in which lime may be abstracted from his natural diet and yet no interference imposed upon all the other elements of his normal food supply. And then, of course, lime nutrition might be restored by simply letting go of the restrictions of the lime-freed diet and allowing the dog to go back to his bones, shift for himself, and fare as he chooses. I am very glad to acknowledge the impetus for this experiment to Russell's generalization, and am also fortunate for suggestions in carrying out the details of this régime of lime starvation in dogs, from the beautifully thorough and seemingly forgotten work of Voit,* published over thirty years ago. Voit produced rickets in dogs by protracted periods of lime starvation, although without any final calcium restoration, as in our experiments, and gives a most exhaustive gross description and analysis of osseous changes.

Accordingly, on May 1, 1911, six puppies in two litters of three each were subjected to lime starvation, except one exempted as a control. These five puppies were locked up in cages and fed on selected, raw, chopped lean beef, mixed up with pure "leaf" lard and distilled water. The control puppy was allowed to eat whatever he chose from the waste

* Voit, Edwin: Zeitschrift f. Biologie, vol. XVI, 1880, pp. 55-118.

of the hospital kitchen—milk, oatmeal, soft bones, meat, and the like. From time to time the lard and meat were cooked to change its monotony. These young dogs had just been weaned, and were perhaps six weeks or two months old. At the end of seventy-five days it was surmised that lime starvation must have reached some considerable degree in the tissues at large, for the dogs had grown and thriven. And as growth and stature are largely due to increase, lengthening, and development of bone, the growing bones in these dogs must have seized upon and absorbed any residual store of lime in their tissues, since there was practically no intake of lime, though abundance of every other essential element of food. Lime forms 85 per cent. of bones.

So the experiment at this point seemed to fit the artificial conditions planned out; namely, with no interruption of tissue growth in all other respects, there has been contrived and carried out a dearth, a poverty, a using up of lime in the tissues and of its storehouse of supply for their use. In the meantime three puppies, two of one litter and one of another, died from some unknown cause. There were then left at the end of seventy-five days three dogs, two lime-starved, and the control dog, which may be designated respectively A, B, and C.

At this point it is well to emphasize the fact that these dogs suffered no general starvation, but were deprived of lime and lime only. The criticism was offered that other complicating and unknown parts crept into the project, and left the truly remarkable effects of lime starvation on tuberculous infection open to the suspicion of some interloping or marplotting factor. But this, it seems, is not so. Indeed, the terms of the experiment are such that the elimination of these surreptitious factors is precisely what was designed and accomplished. The first duty of a purposive experiment is to cut out complicating issues and narrow down the interplay of such factors as we desire to control or get an answer from, and as are pointedly relevant to the matter in hand.

At the beginning of the experiment they weighed three to three and one-half pounds. At the end of seventy-five days the two lime-starved dogs weighed seven and seven and one-half pounds, and the control animal five and one-half pounds. No just comparison in this contrast of weights can be had, because the control dog, belonging to another family, may have been naturally undersized or come from a smaller breed. But the contrast at least tends to show that the two heavier dogs were neither stinted nor stunted, starved nor impoverished nor weakened, except in one particular—the deprivation of lime. And, moreover, in spite of this, they grew up seemingly well nourished and sound. So if there are modifications, departures, or interruptions in the course of the ensuing tuberculous infection, these are to be ascribed to lime deprivation and restoration and to this alone.

So little did lime starvation betray itself that you could not have told these dogs from any others. There was nothing noticeable about them, but in general they appeared a trifle weaker than their normal companion. And, although larger, when they played or quarreled with him they rather got the worst of it, stumbling and being unsteady on their feet.

It is quite likely, however, in any endurance test or a long run, the large-sized dogs would have fallen behind. As a matter of fact, these dogs had a certain degree of rickets, and an X-ray photograph exhibited the rachitic rosary of beaded ribs not present in the control and companion dog, although there are no other contrasts or deformities in the skeleton. One provision must be made here. I cannot say how faithfully my assistant carried out this lime starvation régime or how thoroughly and completely these dogs were starved of lime. I suspect that from time to time it was easier to interrupt the diet, without my knowledge, than to carry it out. Indeed, from subsequent experiments, I suspect that the dogs were not completely starved, and that from time to time in one way and another managed to get the food they craved. If this is so, it speaks all the more strongly for the experiment, for, of course, it means that the degree of lime starvation was not gross, exaggerated, or extreme.

And now for the bacilli. These were of the virulent bovine type, and two weighed milligrams of them in two centimeters of salt solution were injected in the jugular vein of all three dogs on July 15, 1912, in precisely the same amounts and volumes and with the same rapidity of injection. The next part of the plan was to continue the lime starvation in one dog, restore it in the other, and watch the effects in their behavior side by side, counterbalanced by the normal dog with his infection. So, after ten days, in order to give the bacilli time to lodge and take hold, dog B was given back his natural food, while A was held down continuously to his privations in lime-free food. Thus B was given soup and other bones, milk, oatmeal, cooked meat, and, in general, the waste of the kitchen. B was put side by side with the normal control dog and the two fared alike, while their companion in the lime deprivation hardship continued with the selected lean, raw, rump beef, lard, and distilled water.

In some forty days a great contrast began to be apparent. The continuously lime-starved dog began to fail. He became sick and weak, and he began to emaciate and lose weight—2½ pounds. In the succeeding four days all of these manifestations increased and grew worse, and finally, unable to stand up any length of time, he laid down on his side most of the time and was found dead forty-four days after the inoculation.

But while the continuously lime-starved dog was wasting away and dying of tuberculosis, his companion with restored lime nutrition was gaining weight, running and jumping about in the animal yards without the slightest embarrassment from the inoculation or any other trouble. And this was the case also with the normal dog. Indeed, at this time the remaining two dogs had nearly doubled their weights. Thus, on August 28, 1911, when the continuously lime-starved dog died forty-four days after the inoculation, his companion B had increased from 7 to 11¼ pounds, and the control dog from 5½ to 9¾ pounds. Two weeks later, on September 11th, or fifty-eight days after the inoculation, and forty-eight days after the return of this lime assimilation, dog B weighed 12 pounds and the normal dog, C, 10 pounds.

At this date, from experience with experimental canine tuberculosis, one could feel quite sure that these remaining two dogs had gotten the

upper hand of the infection and would have lived out their natural span of life and died of other causes. But, curious to see what was happening in his tubercles, paralleling such a contrast to his wasted and dead companion, B was despatched on this date, and with him the normal dog, C, so as to compare the experiment with the behavior of the bacilli in a dog whose lime nutrition had never been disturbed in any way.

Now we are ready to look at the organs of these animals, and then later, under the microscope, to contrast and compare the character and course of the reasons.

But first it may be well to be on guard against a misconception which still seems to arise occasionally, especially in the consideration of the lesions in miliary and experimental tuberculosis. And this is the estimation of the havoc of tubercles by wholesale attention to their quantity and distribution to the exclusion of their quality and course. In this way the healing and overcoming power of the body is not always appreciated if the number, size and distribution of the tubercles were made the only and insistent point of distinction. In fact, for the moment it might be well to utilize this criticism, for it will serve to show that in the beginning of the infection no favor was shown in the distribution of the bacilli. And it shows especially also that ten days after the jugular inoculation of the two milligrams of bacilli at the turning-point of the lime restoration, the victor had no handicap over the vanquished dog in fewer lodging points of the bacilli or with fewer tubercles against which he had to contend. Thus, again, no underground or interloping factor in the caprices of bacillary distribution played any hand to tamper with the results.

So with a casual observation of the general tubercular infection it would have been hard to distinguish the fatal case from the recovering dog. Thus the lungs of the two dogs contain, say, in each lobe, several hundred, or, all told, some 3,000, miliary tubercles. The livers were greatly enlarged, weighing in each case 580 grams, and were literally riddled with miliary tubercles. In fact, the livers were so involved with tubercles that collectively they were almost of the weight and volume of the organs themselves. You can picture the scene of the liver involvement by imagining a tubercle for nearly every other liver lobule and just about its size—a pin's head. Tubercles in the spleen in either dog were not visible.

With the exception of minor difference in the lymph glands, there is nothing much further to relate in the distribution in the tubercles in these two dogs. As forecast in the X-ray photographs, there were no gross changes in the skull, vertebral column, or bones in general except the ribs. These, in both dogs, showed a string of pearly knots at the junction of the cartilage and the bone—the rachitic rosary. At first the skull-cap appeared thin and the ridges between the brain convolutions livid and finely roughened, like emery paper, but comparisons with various dogs of the same age make this observation dubious, and put it in that form of personal equation which blurs the observation of what actually occurred with the anticipation of what was expected. So, also, rather more changes in the ends of the long bones were expected than actually came to pass,

and yet the foot of the femur and the head of the tibia in each dog appeared more fragile than usual. They split open easily. There was very little covering to the head of the bone, and the line of junction with the shaft exhibited the jagged, bluish-white zone described in rickets.

It is now in order to take up the critical part of the experiment in the contrast of the character and development of the tubercles. Herein the differences are truly striking, and they show best in the liver. Necrosis —cellular death—is the sinister sign in the tubercle; connective-tissue metamorphosis, the hopeful one. In the one it is the rule to find virulent bacilli ready to commence their depredations elsewhere, but in the other it is equally the rule to find the bacilli scarce or absent, and, such as there are, locked up and out of harm's way behind the guardian walls of connective tissue. Thus we speak of open and closed tubercles. And the turning-point, it seems, in the whole career of the tubercles and their possessor is this development of connective tissue. What is back of this great deciding factor of connective-tissue metamorphosis and what spurs its growth? This would appear to be a very vital and relevant issue in tuberculosis, which, unfortunately, tubercle bacillus research does not answer nor even consider. In the instance of our dogs this connective-tissue subjugation of the tubercles appears to go hand in hand with competent lime assimilation.

In the liver of the defeated dog the tiny pinhead tubercles project above the surface. They seem swollen and to be distended with something. In the victorious dog they are flush with the surface, and perhaps they even dip beneath, like little shallow pits or dimples. In the former case, too, the nodules are translucent. With an eight-fold magnifying glass one can see inside of them and into their contents. But in the contrasting liver the little islands are quite opaque and solid. And, moreover, delicate little embroideries and fine traceries of connective tissue run out from their corners and angles. With the hand-glass, especially in the tubercles of the first liver, the little clouds of Naples yellow, necrotic material occupy the centers and even fill up most of the nodules. But in the tubercles of the second liver there are no such peculiar yellow centers and curds. The most striking difference, perhaps, is the contrast in consistence. The liver tubercles in the fatal case are soft, like so many little bags or cysts, with pasty or only semisolid contents in the most fragile and delicate of walls.

Indeed, the walls seem so frail that they appear to be formed more by the surrounding liver tissue than by any intrinsic envelop of the envelop itself. In the surviving dog the little islands are dense, hard and solid. Thus in attempting to crush out and smear these latter tubercles they acted like so many little seeds or bullets. Indeed, all that could be gotten on this slide in the smear were little wads of gristly material that could not be flattened out and were so tough that slides were broken at times in their attempted manipulation. This, by the way, is a good test and method to recognize the recovering form of tubercle in general, and especially where the eye fails to find them, as, for instance, in the spleen. And they may be easily told from the advancing soft and necrotic tubercle.

In contrast to this, the hepatic tubercles of the vanquished dog smeared out with the greatest ease and gave a uniform film.

The two sets of smears show a remarkable antithesis as to the bacilli. While there are myriads in the one, none at all could be found in the other. In the lungs the differences of the tubercles were less striking. Indeed, in each dog they appeared much the same; little, translucent, dull, opal-like, pinhead nodules.

The microscopic picture of the livers of the two dogs, with the continuous and interrupted lime assimilation, will have been anticipated. Microphotographs of sections of each, which, unfortunately, cannot be reproduced here, show the contrast at a glance. In the continuously lime-starved dog the tubercles are wide open and in full blast. These tubercles are naked, that is, they lie right against the liver cells, with no limiting membrane whatever; indeed, not the least attempt at one. They are full of cheesy liquid, or necrotic material, with a loose rim of small round cells.

But in the liver of the surviving dog there are walls of connective tissue and fibroblasts. Each has this little shell or capsule, and, had we allowed the dog to live, these probably would have become thicker and thicker, and would have advanced more and more to the center and have obliterated the tubercle. The cheesy centers also are shrunken and seem to be on their way to disappearance—absorption—however this occurs. To what is this wonderful metamorphosis due? The answer seems to be, forty-eight days of lime assimilation. How did this operate? We do not know.

The differences in lung and spleen are not so pronounced. The process of healing has been more tardy in these two organs. It must be remembered, too, that only forty-eight days had elapsed in the return of lime assimilation in the successful dog, and that the healing process in tuberculosis is from its nature slow, and goes on little by little and step by step. And yet, while the contrast is not so pronounced as in the two sets of livers, the tubercles in the victorious dog in its spleen and lung are more compact and denser than in his companion. In the former two there are fewer individually necrotic cells and a larger number of fibroblasts, although these are scattered about on the edge of the tubercle and not banded together in a zone or wall, as in the liver.

Now, while this experiment seems to answer some question about lime starvation and tuberculosis, it presents a good many subsidiary ones, and we have only begun with this line of experimentation.

There remains the contrast of the perfectly normal dog, inoculated with the same bacilli, on the same date, and killed on the same day as the restored lime assimilation dog fifty-eight days later. In general, to be brief, this dog had not the hundredth or even the thousandth part of the quantitative involvement of the other two dogs. The lungs were perhaps as badly involved numerically, but in the liver there was only a fringe of miliary tubercular involvement about an inch along the borders. This dog's liver weighed only half as much as those in the other two animals. So, then, the lime starvation induced a worse involvement of

tubercles quantitatively. Then, again, the tubercles in this dog were like those in B, healing, and especially in the liver encapsulating on their way to connective-tissue obliteration. As in B, also, the bacilli were absent or exceedingly difficult to find. What happened then in this experiment was that the natural resistance and refractoriness of dogs to tuberculosis were taken away and returned just as we chose in the deprivation or restitution of lime assimilation. It is customary and useful to sum up at the close of an article and present conclusions. But they seem obvious and perhaps are worth attention without especial encomiums and reinforcements.

BRIEF NOTES ON TWO SETS OF DOG EXPERIMENTS FROM VAN GIESEN'S INVESTIGATION ON LIME METABOLISM AND TUBERCULOSIS *

Set I.—Dogs I, J, K and L.

Dog I, born May 18, 1912, was used as a control. It was normally fed with milk, bones, meat, etc. On September 24, 1912, inoculated in the jugular vein with 2 milligrams of Ravenel culture of *B. tuberculosis;* weight was 8½ lbs. Died unexpectedly on November 6, 1912 of tuberculosis. The dose of 2 milligrams of such a virulent culture proved to be excessive for this small young puppy.

Autopsy.—The lungs and liver were studded with pin-point opalescent miliary tubercles. The microscopic examination of the liver showed it to be full of microscopic tubercles, a large number of which were distinctly encapsulated; a few showed no capsule, although most of them showed a tendency towards connective tissue formation; in some places the tubercles coalesce; none are cheesy, all consist of aggregations of small round cells with numerous giant cells. This normally fed puppy was overcome by a large dose of this extremely virulent culture, although there was an apparent effort at healing its tubercles by fibrosis.

Dogs J, K and L were lime-starved dogs. Up to August 14, 1912, their diet had been normal; dog J was about 3 months old and weighed 5½ lbs.; dog K and dog L were about 10 weeks old and weighed 4½ and 5⅛ lbs. Then all were started on the raw meat, leaf lard and distilled water diet. Their weight on September 24, 1912, had increased (dog J weighed 11½ lbs.; dog K 7¾; dog L 7½ lbs.), when all were inoculated in the jugular with 2 milligrams each of the Revenel strain of B. tuberculosis.

The lime-free diet was continued but had to be relaxed with an occasional small amount of milk.

Dog L died about October 15, 1912, weight 5⅛ lbs. Dog J and Dog K died October 30, 1912, weight 9¾ and 6⅞ lbs., respectively.

Autopsy on Dog J.—Lungs and liver were riddled with pin-point opalescent miliary tubercles. Ribs were not beaded, but bulged out a little at the junction of the cartilages. A retroperitoneal cheesy gland,

* These notes have been compiled by Dr. Irma Le Vasseur who assisted Dr. Van Gieson in these investigations.

the size of a chestnut, was found. The liver on microscopic examination, showed minute tubercles throughout the field, which were not caseous nor encapsulated, but opening freely in the liver tissue; the liver cells were greatly degenerated and full of vacuoles.

The lungs and livers of dog K and dog L also were studded with miliary tubercles. Their ribs were markedly beaded. The liver of dog K, under the microscope, was largely composed of coalescent tubercles, in which no attempt at calcification appeared; the centers of the tubercles were composed of very much disintegrated cells but not caseous. The liver of dog L also was full of microscopic tubercles (islets of small round cells) non-encapsulated, non-caseous; no sign of connective tissue formation whatever.

Set II.—Dogs X, "Alpha," "Beta" and "Mutt."

The last one—the control—was thrown away by mistake after death before a post-mortem had been performed. Dog Beta being exactly like dog Alpha in weight and behavior, no tissues were preserved. Dog K and dog Alpha were studied.

Dog X was received October 4, 1912, at the age of 5 weeks. Normally fed until December 6, 1912, when it was put on the raw meat, lard and distilled water diet. Injected intravenously on December 19, 1912, with ¾ milligram of the Ravenel culture; weight was 18 lbs. The lime starvation was kept up until January 9, 1913. Death occurred from tuberculosis February 2, 1913; its weight was reduced to 12¾ lbs. Twenty days of lime-free diet following immediately the inoculation probably favored the bacilli in securing a stronger foothold in the tissues, so that not much benefit was derived from the normal diet and lime assimilation during the twenty days preceding death.

Autopsy.—Miliary tuberculosis of the lungs; the tubercles were translucent and would not readily spread when smears were attempted. Perilobular tuberculosis of the liver. Abundant bacilli were found in smears of lungs, liver, spleen, and an indurated sternal node, the size of a bean.

Microscopical Anatomy.—Most of the liver tubercles were encapsulated and showed connective tissue trabeculae traversing them. Not much liver tissue remained, mostly replaced by non-cheesy tubercles; a few of the tubercles had no limiting membrane, and showed no attempt at calcification.

Dog Alpha was received with dog Beta October 19, 1912, when six weeks old. Put on raw meat, lard and distilled water on December 6, 1912. Each animal was inoculated intravenously December 19, 1912, with ¾ milligram of Revenel culture; the weight of Alpha was 9 lbs., that of Beta 10 lbs. Both died January 8, 1913, Alpha weighing 8 lbs., Beta 8¼ lbs.

Autopsy (both puppies).—Miliary tuberculosis of the lungs and liver; abundance of bacilli in smear of lungs, liver and spleen. The microscopic examination of dog Alpha's liver showed it to be studded with microscopic tubercles, non-caseous, non-encapsulated.

Conclusions

These two sets of experiments seem to corroborate Dr. Van Giesen's first findings in dogs A, B and C in that the healing process of tuberculosis by connective tissue formation is dependent upon the lime intake. In the above six dogs the livers of Dog I, which was normally fed, and dog X, which was lime-starved but had normal food restored later, showed an apparent attempt at healing of the tubercles by fibrosis while in striking contrast with those findings, the lime-starved dogs J, K, L and Alpha showed tubercles which were devoid of any connective tissue reparative formation.

Note.—Dr. Van Giesen also had under way an extensive study upon bone lesions in tuberculosis. The investigation, in part, considered a possible action of the toxins of the tubercle bacillus upon the osteoclasts, stimulating them to extract lime salts from the bones which might aid in the calcification of the tubercles. The large material collected included various bones, normal and tubercular, from different species of animals taken at different ages. If possible this work will be continued, and reported upon later.

CONCERNING THE CULTIVATION OF THE RABIES ORGANISM

Anna Wessels Williams

From the earliest days of artificial cultivation many investigators have attempted to grow the specific organism of rabies, but few have had the courage to report positive results. Among the latter may be mentioned, for example, Bruschetti (1896), who reported that he had cultivated upon special brain-broth media an extremely minute pleomorphic bacillus which up to the *sixth* culture generation produced typical rabies in susceptible animals; and later Levy (1903), Sormani (1903), and a few others reported equally positive results with cultures of different micro-organisms isolated by them from rabies animals. No one so far has been able to corroborate these results.

Since Negri and others demonstrated that the structured cell inclusions known commonly as *Negri bodies* are specific for rabies there have been practically no cultural studies reported, but from verbal reports and demonstrations we know that such studies have been going on in several research laboratories.

Among those most interested in the subject is the Research Laboratory of the New York City Health Department, which has been more or less actively engaged in investigations concerning the etiology of rabies since 1897, when practical studies on the treatment of this disease were begun there.

As a culmination of a series of studies on the etiological side of this question I published several reports in 1904[1] and 1906[2, 3, 4] dealing chiefly with the nature of the Negri bodies in which it was shown that the evidence presented was strongly in favor of a specific protozoal cycle of development in these bodies. I, therefore, wisely or unwisely, gave them a generic and specific name, *i. e., Neurorrhyctes hydrophobiae.* The chief points first brought out in these papers which led to this conclusion, may here be briefly recapitulated.

1. By a new method for the examination of rabies brain (the smear method) Negri bodies show distinct characteristics in both morphology and stain.

2. Their morphology is constantly cyclic, *i. e.,* a definite series of forms indicating growth and multiplication can be demonstrated; small, single, rounded or oval plastin-staining forms containing one to several minute chromatin-staining granules; similar forms in twos or groups; larger forms containing a definite central or eccentric mass (nucleus); forms with smaller chromatin masses arranged in a ring about the central mass; evidence of division of these larger forms as well as of the smaller ones; segmentation of chromatin and distribution of nuclear-staining material throughout the whole organism; division of the organism into many minute bodies; and finally, from the beginning of the appearance of the smaller masses of chromatin, all stages of *budding,* a

phenomenon which accounts for the appearance in the same cell and at the same time of both large and small forms, and also helps to account for the rapid spread of the organism and for forms small enough to pass certain filters.

3. In rabbit "fixed virus" besides the few larger forms seen by others, very many extremely minute forms are found, within most of which are seen in well fixed and stained preparations a single chromatin granule.

4. With stains such as Giemsa's the lightly basophilic property of the "cytoplasm" of the bodies and the chromatin-like nature of the contained masses and granules are brought out in smears better than in sections.

5. Negri bodies are found in *all* parts of the infectious central nervous system, beginning to appear in the large nerve cells as extremely minute forms before the beginning of symptoms, *i. e.,* on the fourth day in fixed virus infectious and on the seventh day in rabbits inoculated with street virus; thus they were found early enough to account for the infectivity of the host tissues.

Most of those findings were confirmed by a few investigators immediately following their publication. Recently Watson[5] added his corroboration and states that he found in addition spore-like bodies similar to myxosparidian spores.

No one has yet brought forward clear evidence against these findings of a definite cycle of development.

In the last number of the Journal of Experimental Medicine appeared Dr. Noguchi's[6] announcement on the cultivation of the parasite of rabies. This announcement unfortunately leaves us with no more exact knowledge of the cultivation of the rabies organism than we had before.

Naturally, with the idea of the protozoal nature of the Negri bodies in mind many attempts have been made by me to grow them in artificial culture media, but results so far have been either too unsatisfactory or too unfinished for publication. Others have tried newer culture methods also with negative results. Thus Poor and Steinhardt[7] state that they were unable to obtain evidence of growth by the living tissue method of cultivation.

A few suggestive ideas, however, have been obtained from some of the observations made by me which, in order to discuss Noguchi's report, may be published now.

In the first place it may be stated that no change of mind has been necessary concerning the varieties and arrangements of the Negri bodies as shown in my plates published in 1906. Such forms are still called "typical Negri bodies."

The observations may be divided into two groups.

A. *Changes in rabid brains after death of animals.*—When brains containing "typical Negri bodies" are kept free from contamination, either at icebox (12° C.) or room (24° C.) or incubator (36° C.) temperature, certain changes occur, more slowly at the lower temperatures.

First, in rabbit "fixed virus" brains which usually show only the

minute forms (fig. 1, a-d), some of the larger forms appear in about 24 hours at 36° C., 48 hours at 24° C. and 3 days at 12° C. (fig. 1, f-g, i) and more definite groups of smaller forms (fig. 1, e.h.j.). Some of these groups seem to be surrounded by a sort of a membrane taking a mixed chromatin and plastin stain and others seem to be free. These changed segmented bodies remain longer at the lower temperatures and disappear where the brain becomes much softened.

Second, in "street" and "passage" virus brains, many of the large forms appear to become distinctly separated into smaller forms, some within a "membrane" and some in free groups (fig. 2 a-h).

These changes are seen more clearly in brains left in the skull of the animals or in brains excluded from the air by some method such as pouring agar or serum over them. Animals sent in for diagnosis, which have been buried for some time during cool weather may show these changes quite distinctly.

By animal inoculations with dilutions, such material seems to have gained somewhat in virulence. Whether it is a real gain by an actual increase in the number of organisms, or whether higher dilutions are more virulent simply through the breaking up of groups of organisms allowing more even distribution throughout the diluent has not been decided.

B. *Passage of virus from culture to culture.*—The last cultural method tried is a modification of the tissue-media method employed by Williams[8] in obtaining pure cultures of amebas from amebic dysentery.

A thin slice of fresh sterile guinea pigs brain was placed on freshly made protozoan agar plates over a piece of rabbit fixed brain the size of a pea. Over this mass was then placed a thick layer of protozoan-agar. This was kept at about 25° C.

Transplants were made at the end of seven days by removing the agar, stirring up the mass, transferring about one-fourth of it to a fresh agar plate and covering with fresh brain tissue and agar as before. After each transplant some of the brain mass (about the size of a pea) was made into an emulsion with 5 c. c. normal salt solution, and two drops of this emulsion were inoculated intracerebially into guinea pigs. The pigs came down with typical rabies after the inoculation of the fourth culture generation in all of the three series so far tried. By the fifth transplant in these series the material was contaminated, and the inoculated animals did not come down with rabies.

The question of dilution of the virus was of course considered, but here again the controls have not yet been sufficient to be sure that some growth, however slight, did not take place.

The larger apparently broken up Negri bodies (fig. 1, e-j) could not be found except in cases where the possibility of having a piece of the original brain material in the smear could not be ruled out.

Of course, in each brain mass "pleomorphic chromatoid granules" arose, but so did they in the controls, and no difference could be determined between them except that they were in somewhat smaller numbers in the controls.

Noguchi says that in culture media similar to that employed for the cultivation of the spirochaetae of relapsing fever (are we to infer from this that it was the method he found *best* for these sporochaetae, or one of the others or all?) "very minute granular and somewhat coarser pleomorphic chromatoid bodies arise which on subsequent transplantation reappear in the new cultures through many generations."

Here is nothing definitely new. From Pasteur down nearly all investigators on this subject have stated at one time or another that minute and coarse granular pleomorphic bodies are the cause of rabies, and any observer may see granular bodies answering to Noguchi's description, arising in uninoculated tissue media after the tissue has undergone a certain amount of degenerative change.

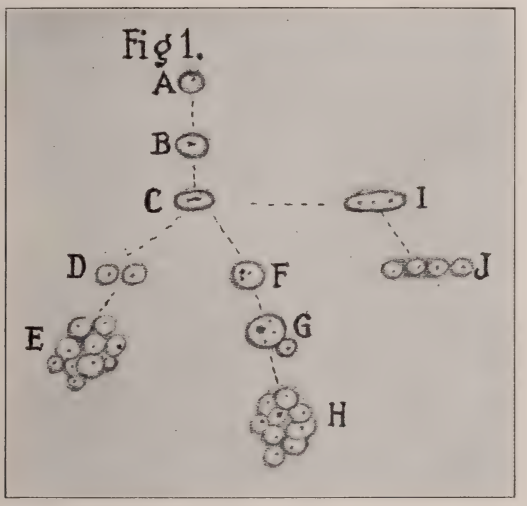


FIG. 1.—From rabbit "fixed-virus" brain; *a, b, c, d, f* and *i*, types of Negri bodies seen at death of rabbit; *e, g, h* and *j*, apparent multiplication and segmentation of the bodies after three days at 24 C. All drawings in this illustration and Figure 2 made from smears stained by Giemsa's method and magnified about 2,000 diameters.

Noguchi continues, "on four different occasions I observed in the cultures from 'passage' and 'fixed' virus nucleated round or oval bodies surrounded with membranes totally different from the minute granular bodies although arising in the cultures in which the latter occurred." Is it certain that these bodies are not segmented bodies from the original material carried over?

No mention is made of culture generations in the body of Noguchi's article, but in the description of the accompanying plate it is noted that two of the observations were made on "a culture (second and fourth generations, five days old)."

In regard to the specific virulence of his "cultures," Noguchi says: "By inoculating cultures containing the granular, pleomorphic or nucleated bodies, rabies has been produced in dogs, rabbits and guinea pigs."

Here again, the culture generation is not stated. Furthermore, as nothing is said about the amount of material transferred, or about the number of days before the transfer is made or about control dilutions in testing virulence the question may be raised as to whether the positive results asserted were not due to dilutions of the original material rather than to continued growth and division of a specific micro-organism.

It is hoped that later reports by making clear these details may help decide this question.

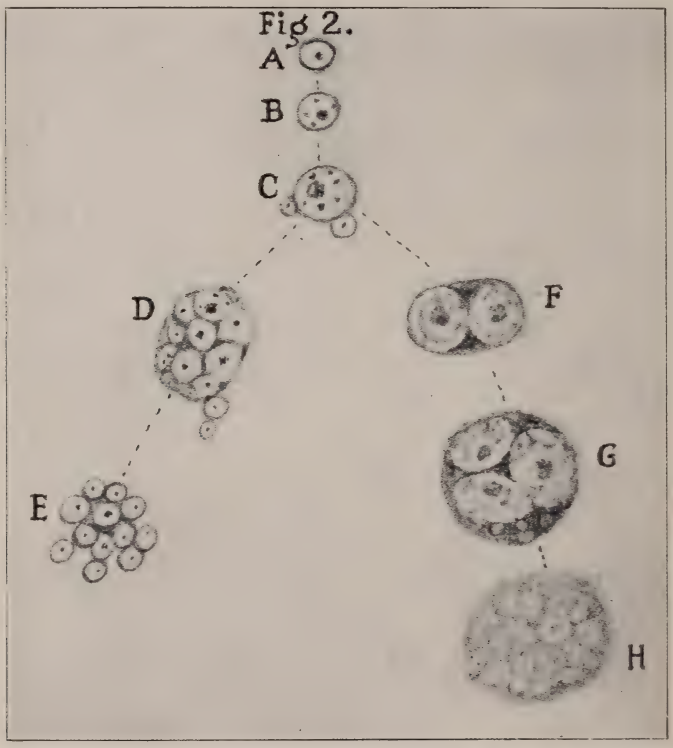


FIG. 2.—From dog "street-virus" brain; *a*, *b*, *c* and *f*, types of Negri bodies seen at death of dog; *d*, *e*, *g* and *h*, apparent multiplication and segmentation of the bodies after three days at 24 C.

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THE ACTION OF GASES IN THE CIRCULATORY APPARATUS

Charles B. Fitzpatrick and James P. Atkinson

Little is definitely known about the action of gases in the blood vessels. This is especially true in its relations to sudden death.

According to the general experience of investigators upon this subject, a dose from 60 to 100 c. c. of air given at one time usually constitutes the fatal dose, causing death almost instantly. Ninety-five cubic centimeters given intrajugularly in our experiments with normal dogs generally caused death almost immediately. After a number of experiments with the previous introduction of glycerin or olive oil into the femoral vein, to prevent foaming or churning after introduction of large quantities of air, we have found that the use of these substances is not necessary. We have obtained as good results without the use of olive oil or glycerin. The details of these and related experiments of interest are briefly outlined as follows:

Experiment I.—An intravenous administration of 3.5 c. c. of a 50 per cent. solution of glycerin in normal salt solution into a 35-pound dog, which was followed by the rapid introduction of 28 c. c. of air into the femoral vein, gave no harmful symptoms or any trace of a kymographic disturbance.

Experiment II.—A dog weighing ten pounds was used to determine the toxic dose of glycerin injected intravenously. The introduction of 7 c. c. of 50 per cent. glycerin in normal salt solution caused an immediate and marked drop, followed by a slight rise in the arterial pressure. This was followed by another equally short drop in pressure, with cessation of heart action. Respiration continued for a time then quite suddenly ceased.

Experiment III.—Dog, thirty pounds. This dog was given 3 c. c. of 50 per cent. glycerin in salt solution in doses of 1 c. c. and 2 c. c., respectively; about three minutes after the administration of the glycerin 85 c. c. of air in 5 c. c. doses were injected into the femoral vein during the next seven minutes, with no noticeable effects save a slight rise in blood pressure immediately following each injection. Five minutes later 50 c. c. more were given in the same way; this produced a slight fall followed by a rise and fall in the pressure. A return to normal took place within thirty seconds. This injection was followed in thirteen minutes by 45 c. c. of air in the same way with similar results. The total amount of air injected was 180 c. c. This was followed in two minutes by 25 c. c. (215 c. c. in all) with similar results, *i. e.*, slight fall in pressure with immediate recovery. Forty-five cubic centimetres of air after two minutes rest were given in the same way during a period of sixteen minutes. The total amount of air injected thus far into the femoral vein of the dog was 260 c. c. The jugular vein was next opened and 250 c. c. of air were then injected into it during the following twelve minutes. This made a total of 310 c. c. of air introduced without evil effects, in-

deed the animal seemed to improve and often came out from under the ether. A cannula, one-eighth inch in diameter, was next introduced into the left external jugular and the vent left open. No noticeable amount of air was drawn in and the condition of the animal remained the same. The vent was left open several minutes, and then the animal was instantly killed by the operator blowing his breath through the cannula into the jugular.

Experiment IV.—One cubic centimetre of a mixture consisting of equal parts of glycerin and physiological saline solution was introduced into the femoral vein of a thirty-pound dog (brindle, mongrel bull) without any noticeable vaso-disturbance. 200 c. c. of air were then insufflated into the femoral vein during the following ten minutes in twenty doses of 10 c. c. each. The insufflation caused a slight rise followed by an extremely slight fall in the vaso-pressure to follow each dose. 3 c. c. of the 50 per cent. mixture of glycerin in physiological salt solution were then injected into the femoral vein. This was followed by the insufflation into the femoral vein of 40 c. c. of air, three minutes after the previous administration of 200 c. c. within a period of three minutes, making a total of 240 c. c. No evil effects were indicated, the animal completely recovered from the shock and was apparently coming out from under the ether. 190 c. c. of air were then insufflated into the femoral vein within the next period of five minutes. The dog recovered completely from this insufflation of air and died later apparently from ether poisoning. The total amount of intravenously administered air given to this dog amounted to 430 c. c.

Experiment V.—A 50-pound dog was given 5 c. c. of 50 per cent. glycerin in three equal doses at intervals of about one minute. This dog received 100 c. c. of air into the femoral vein after four minutes had elapsed, during a period of three minutes, in 5 c. c. doses, without any noticeable effect; 100 c. c. more were given in the same way during the next three and one-half minutes, with no bad effects. Two hundred cubic centimetres more were given during the next four minutes with no evil results. 200 c. c. were again given during the next four minutes with no bad effect. This made a total of 600 c. c. The action of the heart continued good and then became slowed and the respiration deepened. The heart continued slow and there were four attacks of slight arterial depression. The blood pressure and the respiration returned in fifteen minutes to about as good a condition as at the beginning of the experiment. This dog was killed by repeated doses of commercial kerosene administered in doses of 5 c. c. in an attempt to find the fatal intravenous dose of kerosene. The first 5 c. c. caused a moderate drop followed by a rise. In 30 seconds after the first dose 5 c. c. more were given and a gradual fall took place. This fall continued during each of the following doses of 5 c. c. which were given at intervals of 30 seconds. Eleven doses were given in this way before the heart stopped, followed by cessation of respiration one minute later.

Experiment VI.—A fifteen-pound normal, mongrel dog was etherized and given an intravenous injection of 2.5 c. c. of olive oil mixed with 2.5

c. c. of physiological salt solution followed in three minutes by the introduction of 5 c. c. of air into the femoral vein without any noticeable effect. At the end of the next six minutes somewhat less than a 100 c. c. were insufflated into the femoral vein; at the end of the next three minutes 100 c. c. more were injected. This was repeated at the end of each following three-minute interval, seven times, making a total intravenous sufflation (allowing for leaking) of somewhat less than 900 c. c. within thirty-three minutes. At the end of the next period of three minutes 5 c. c. of a 50 per cent. mixture of olive oil and physiological saline solution were injected into the femoral vein. This was followed in one minute by 3 c. c. more of the same 50 per cent. saline mixture of olive oil. A small cannula needle (⅛ inch in diameter) was then introduced into the left external jugular and at the end of two minutes after the last oil injection 70 c. c. of air were insufflated into the jugular within one minute; 30 c. c. more were insufflated into the jugular within the next thirty seconds. No marked effects followed these administrations. At the end of the next minute 100 c. c. more of air were insufflated into the left jugular during a period of thirty seconds. Excessive panting followed this, but the animal appeared strong and otherwise well. He would have recovered to all appearances, if left undisturbed. The total volume of air insufflated thus far was 1,105 c. c. At the end of the next interval of one minute, 100 c. c. of air were again insufflated very rapidly within thirty seconds, and in one minute following its insufflation the animal after giving vent to a cry of distress with labored breathing, stiffened and died still under the influence of ether. An occasional respiratory gasp followed at intervals of about from 45 to 60 seconds during the next few minutes. The total amount of air given intravenously to this animal amounted to 1,205 c. c.

Experiment VII.—A normal mongrel dog, weighing fifteen pounds, was used as a control upon the work of previous investigators, to ascertain the maximum fatal dose of air when introduced into the jugular of normal dogs untreated by injections of glycerin or oil. The dog was put completely under ether, and then the small cannula needle was introduced into the left external jugular, and then within a period of one minute 60 c. c. of air were insufflated. The animal panting and gasping appeared to be dying. The insufflation was continued and 30 c. c. more of air were insufflated, at the end of which administration the dog was dead. A few gasps followed at intervals of from thirty to sixty seconds during the next few minutes. The fatal dose of air insufflated into the jugular continuously then is apparently somewhat less than 60 to 90 c. c. in this type of dog. The air was introduced by means of Hempel's gas burette.

Experiment VIII.—With a view of controlling the results following the prior administration of olive oil solution, we did the following experiment: A normal mongrel spaniel dog, weighing eighteen pounds, was etherized and received about one-half hour later 5 c. c. of a fifty per cent. mixture of olive oil in physiological saline solution, into the left femoral vein. One minute later 2.5 c. c. of the same mixture and

then again 2.5 c. c. of it, after an interval of one and one-half minutes, were injected. We waited five and one-half minutes and then insufflated through a cannula 1/16 of an inch in diameter, which had been introduced one and one-half inches into the left external jugular and firmly ligatured in position, one hundred cubic centimetres of air, measured by means of Hempel's gas burette during a period of one and one-half minutes. The heart stopped at the end of this period and the respiration almost immediately ceased. The animal made no panting or other sound. A few gasps followed during the next three minutes and the animal was dead.

We believed at first that glycerin and olive oil gave an increased tolerance for the presence of air in the blood stream. But the experiments upon the untreated dogs show these previous injections of glycerin or oil to be unnecessary.

A STUDY OF ICTERUS NEONATORUM BY MEANS OF THE DUODENAL CATHETER

Alfred F. Hess

The occurrence of jaundice within the first few days of life has, as might be expected, stimulated repeated investigation. The theories advanced to account for this striking phenomenon have been most ingenious, but being based quite as much on speculation as on fact, they have proved unsatisfactory and have been accepted only tentatively in lieu of something sounder. The excuse offered for entering on a path so well trodden is that a new point of vantage of this subject now presents itself, through the use of the duodenal catheter, recently described in quite another connection.[1] By this means direct access to the bile is afforded at its site of excretion. Before presenting the data gathered by this means, however, it would seem of advantage to sketch in outline the present status of this question.

It is to be noted at the outset that there is a surprising lack of unanimity even as to the incidence of icterus neonatorum. This is all the more remarkable when we consider that this phenomenon is of so simple a nature that it should not allow of a wide variance of opinion. Nevertheless we find the incidence given by some as 15 per cent., by others as 85 per cent., and by still others placed at various levels between these extremes. This marked divergence must be attributed almost entirely to differences in method of examination. It would seem that those who made a point of examining with the aid of strong daylight, and of looking for the faint yellow tinge on the back or on the nostrils, rather than in the sclera, as in the case of adults, have observed icterus in at least 75 per cent. of infants. In a series of 100 infants whom I examined for this sign, it was definitely noted eighty-three times. But even this figure probably does not give us a correct idea of its frequency, for it is manifestly impossible for us to detect by means of mere clinical inspection the presence of a trace of bile in the blood or in the skin, and when we consider the crudeness of the test, and remember that bilirubin crystals frequently have been found in the tissues of infants who showed no jaundice during life,[2,3] we probably do not err in believing that there are few instances in which bile does not enter the general circulation soon after birth. In view of its frequency, icterus neonatorum must therefore be considered a peculiar entity, the sole example of a physiologic icterus.

The opinion that this icterus is hematogenic in origin, that the bile is formed directly from the blood pigment, does not need more than passing mention, since it was proved experimentally by Minkowski and Naunyn[4] and by others that if the liver is extirpated jaundice cannot be induced; and since Birch-Hirschfeld[5] demonstrated bile acids in the pericardial fluid

[1]Hess, A. F.: Am. Jour. Dis. Child., March, 1912.
[2]Neumann, E.: Virchows Arch. f. path. Anat., 1888, CXIV, 394.
[3]Orth, J.: Virchows Arch. f. path. Anat., 1875, LXIII, 446.
[4]Minkowski and Naunyn: Arch. f. exper. Path u. Pharmacol., 1885, XIX, 34.
[5]Birch-Hirschfeld: Virchows Arch. f. path. Anat., 1882, LXXXVII, 1.

of infants who had manifested icterus neonatorum during life. The blood may likewise play a rôle in the causation of this form of jaundice. The presence of bile acids in the body fluids, however, conclusively showed the participation of the liver.

Although the hematogenic theory is certainly insufficient to account for this form of icterus, or indeed for any icterus whatsoever, it has been accorded a position of varying importance by many writers who believe in the hemohepatogenic origin of this disturbance. Hofmeier[6] was the first to advance the theory that in the new-born there is a marked destruction of red blood-cells in the circulation, that in cases of icterus neonatorum this takes place to an exceptional degree, and that hemoglobin thus set free forms the basis for an excess of bile formation in the liver (polycholic or pleiochromic icterus). Knöpfelmacher[7] has failed to find this destruction of red blood-cells and therefore gives this theory no place in the etiology of this form of icterus. However, very recently[8] blood examinations have again been reported to uphold it. In view of the fact that great weight has been attached to this etiologic factor, we should bear in mind that free hemaglobin has never been found in the new-born in the circulating blood. And what is of far greater moment in controverting the claim that this hemolysis is alone sufficient to account for the icterus is the experimental evidence that jaundice cannot be produced by intravenous injections of hemoglobin, and the clinical experience that it does not supervene following transfusion of blood.

Another theory which cannot be passed by without mention, as it prevailed for many years, was that of Frerichs.[9] The distinguished author of "Diseases of the Liver" explained this jaundice by reasoning that coincident with birth, and closely following the circulatory changes in the umbilical vessels, there results a marked anemia and decrease in pressure in the liver capillaries, which in turn leads to a diminished pressure in the bile capillaries, and an overflow of bile into the blood stream. Quite apart from the fact that this explanation is purely hypothetical, it can no longer be given any weight, in view of the marked congestion of the hepatic vessels, in contradistinction to an anemia which has been uniformly found at autopsy of the new-born.

The theory to-day which, according to Finkelstein,[10] finds the greatest acceptance is that of Quincke.[11] This author believes the patency of the ductus venosus to be the deciding etiologic factor. In this connecting link between the portal and systemic venous circulation fails of obliteration at birth, icterus results, as bile passes directly from the meconium in the intestine to the portal vein, and circumventing the liver, enters the inferior vena cava. There are other subsidiary causes which play a part, such as the polycholia, the small amount of bile pigment excreted by the kidneys, and the peculiar nature of the intestinal contents, the bile con-

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[6]Hofmeier: Ztschr. f. Geburtsh. und Gynäk., 1882, VIII, 287.
[7]Knöpfelmacher, W.: Wien. klin. Wchnschr., 1896, No. 43.
[8]Heimann, F.: Ztschr. f. Geburtsh. und Gynäk., 1912, LXIX, 165.
[9]Frerichs: Klinik d. Leberkrankh., 1858, I, 198.
[10]Finkelstein, H.: Lehrbuch d. Säuglingskrankh., 1905, I.
[11]Quincke, H.: Arch. f. exper. Path. u. Pharmakol., 1885, XIX, 34.

taining meconium. As this theory enjoys such wide acceptance, it demands particular consideration, and it is therefore remarkable that although it was promulgated some twenty-seven years ago, so little experimental work has been undertaken to confirm or refute its validity. Quincke himself investigated the condition of the ductus venosus in only five cases, and in these reported on the circumference of the vein rather than on its lumen, which would seem to be the more important factor. Schreiber[12] supported this theory by citing the figures of patency of the ductus venosus published by Elsässer[13] many years ago. However, these figures may well be used against Quincke's hypothesis. In ninety-two autopsies on infants born alive, the ductus venosus was found patent in sixty-five, and in fifty of these where the infant was seven days or more of age, the ductus was patent in thirty-one and closed in nineteen instances. This includes some premature infants, and omits some cases in which post-mortem conditions seem insufficiently described. In the second week of life the ductus was patent in nineteen out of twenty-seven cases, in the third week in eight out of fifteen cases, and also in four of eight dissections made at a still later period of life. It is needless to add that if we use this anatomic study as a criterion, we should not be led to consider this icterus as a consequence of a patent ductus venosus; for if such were the case, icterus neonatorum would be a phenomenon not of the first week but of the first month of life.

It should be remembered that Elsässer carried out this investigation purely for medicolegal purposes. There has been no investigation to correlate anatomic findings of this nature with the clinical occurrence of icterus, and without this link these mere dissections can furnish but inconclusive evidence. It would seem worth while to study the patency of the ductus venosus also in animals and to determine whether it bears any relation to jaundice.

Knöpfelmacher[14] probed this theory by attempting to remove the meconium from the bowel shortly after birth. With this end in view he used repeated high enemata, employing acidulated water in order not to dissolve the bilirubin, and performed four to eight irrigations of this nature. Although this method is ingenious, its results do not seem conclusive; for on scanning the notes of the twenty cases on which this experiment was carried out, it is evident that the bowel was not completely emptied of meconium. In most cases a "milk-stool" did not appear until the third or the fourth day of life, which must entirely invalidate the test. Furthermore, only one case is noted in which jaundice made its appearance later than the milk-stool, and on this point the entire experiment would seem to hinge—the occurrence of jaundice after and in spite of the emptying of the intestine of all meconium. The question does not seem to be open to solution by this method, for it is impossible to be certain that meconium is not still present in the bowel. Indeed, in this connection Kehrer's[15] figures showing that the jundice bears no relation to

[12]Schreiber, E.: Berl. klin. Wchnschr., 1895.
[13]Elsässer: Ztschr. f. Staatsarzneikunde, 1841, XLI.
[14]Knöpfelmacher, W.: Jahrb. f. Kinderh., 1908, LXVII, 36.
[15]Kehrer: Oesterr, Jahrb. f. Pädiat., Wien, 1871, p. 71.

the date of disappearance of the meconium, and the appearance of "milk-stools," and that among his 123 cases, where the yellow stools appeared late there was no increased frequency or intensity of jaundice, seem quite as convincing as Knöpfelmacher's carefully planned experiment.

There are many other theories which might well be mentioned, but we shall limit ourselves to an outline of a group which may be designated as that of bile stasis. It is unnecessary to consider such hypotheses as an obstruction of the bile-ducts by mucus or epithelium (Virchow), or a congenital narrowness of these ducts (Kehrer), or compression of the larger ducts through edema of Glisson's capsule, as post-mortem examination has failed in every instance to confirm them. There are other investigators, however, who believe that this stasis of bile results from inadequate excretion, that the bile is forced out of the intracellular biliary capillaries into the liver cells and then enters the blood stream. Browitz[16] has supported this theory with careful microscopic examinations which have been confirmed by Knöpfelmacher. Neither believes that the jaundice is due to mechanical obstruction, as no tears in the fine bile ducts are found, merely varicosities, but rather to a disturbance of function of the liver cells allowing a reverse current of bile into the blood stream. In stillborn infants Knöpfelmacher[14] describes an increased viscosity of the bile, but in infants of the first few days of life a markedly decreased viscosity with an abnormal distention of the bile capillaries. The viscosity tests were carried out according to Ostwald's method. However, the bile was obtained from the gall-bladder and not from the hepatic ducts, and may have been a fetal secretion. The histologic investigations showing bile stasis are especially welcome, as they do not burden the already overweighted etiology of icterus neonatorum with another theory.

Investigation with Duodenal Catheter

It is possible to pass the duodenal catheter in the new-born infant with as great ease as in infants a few months of age. The technic is the same; however, a 14 (F.) Nélaton catheter is used instead of the larger 15 (F.) catheter. There is a tendency to slight bleeding due to the delicate texture of the mucous membrane during the first hours and days of life, and for this reason aspiration must be gentle.

It seems that the first point to ascertain should be the time when excretion of bile begins in the new-born. Accordingly the catheter was passed on infants a few hours old; indeed, in four cases within two hours of birth. It is advisable from an etiologic point of view to divide the cases into groups, those tested before they had nursed, and those tested subsequent to nursing. The former group comprises fifty-two cases, almost all under 6 hours old, the time when the infant was usually put to the breast. It includes, however, in addition to four infants under 2 hours old, several 12 hours old. The test was carried out generally in two stages, and the catheter was passed into the duodenum in the manner previously described, and after ten minutes of intermittent aspiration withdrawn; it

[16]Brewitz: Wien. klin. Wchnschr, 1900, p. 785.

was then washed out and reintroduced for the same length of time. Dividing the test in this way has the advantage of avoiding an error which might be occasioned by the plugging of the lumen of the catheter with mucus, an event which would of course nullify the test if it occurred soon after the intestine was entered. It is of further advantage, as it happens frequently in the new-born, owing to the pressure of the pyloric or cardiac sphincter, that it is impossible to aspirate viscid fluid, and the bile or intestinal juice is obtained only after the catheter is withdrawn into the stomach or even into the esophagus. For these reasons the two tests furnish a safer criterion. Where any doubt as to the reliability of the test was entertained, aspiration was continued for a longer period.

Bile was obtained but once in the course of these fifty-two tests, and in that instance in small quantity from an infant 2½ hours old (Table 1). It may therefore be stated that in the first half day of life there is rarely an excretion of bile into the intestine. It is possible, had the test been continued for many hours, a procedure which did not seem justifiable, bile might have been oftener encountered. However, the aspirations were sufficiently numerous to warrant the deduction that bile, in general, is not excreted during this early period.

Nineteen tests were made on infants from 12 to 36 hours old. It was found that where marked jaundice was present, in four cases, bile could be obtained, but that where it was absent, in fifteen cases, bile also was absent. Mere statistics, therefore, cannot give a correct idea of the bile excretion of this period, for they will vary according to the number of cases of marked jaundice which they comprise. This is true to some extent in infants 1½ to 3 days old. As the infants increased in age I found bile in increasing frequency; never to the same extent, however, as in the deeply jaundiced infant (Table 1). There is no doubt that in some instances although bile was not obtained, it must have been excreted to some extent into the gut. This was to be expected when we consider that although bile is secreted continuously it is excreted intermittently.

Table 1.—Relation of Age to Excretion of Bile (Duodenal Catheter)

[No. of Cases	Age	No Bile	Bile	Marked Jaundice	Remarks
52	½-12 hrs.	51	1	0	39 cases were less than 6 hours old
19	12-36 "	15	4	4	Marked jaundice in all four positive cases: in others no bile obtained
15	1½- 3 days	5	10	6	Marked jaundice in four positive cases: in other two jaundiced cases bile next day
13	3- 4 days	4	9	6	Jaundice not increasing
12	4- 5 days	2	10	5	Jaundice decreasing
13	6-11 days	2	11	4	Jaundice decreasing
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In this connection we may refer to Table 2, composed of cases in which numerous tests were carried out on the same infants on consecutive days. A survey of this table shows that when bile was obtained it was almost always found in successive examinations, and that its presence was not subject to caprice.

In twenty-four cases a careful study was carried out of the relation of the bile excretion to jaundice. In some of these cases the catheter was employed in a routine manner soon after birth, and then every day subsequently until bile was obtained on two successive occasions. These infants were unselected. In eleven of the cases, however, a selection was made, based on the occurrence of early or marked jaundic. Seventy-two tests in all were carried out, the results of which are given in Table 2 (a) (b). A most striking and consistent fact was that bile was not obtained in any case previous to the appearance of jaundice. Even where bile was obtained within the first twenty-four hours, a marked early jaundice also manifested itself (Cases 9 and 19). It is evident that the quantity of bile secreted plays an important rôle. In one case (Case 3), for example, excretion was delayed, and bile was not obtained until the seventh test, so that unless secretion of bile had been exceptionally meager, this certainly must have resulted in marked icterus. On the contrary, a slight ephemeral jaundice was noted on the second day. And again if we compare a case in which there was no jaundice (Case 4), with another in which there was the most intense saffron jaundice, we find that in the latter instance the excretion of bile began earlier and was incomparably more profuse; indeed, in the jaundiced case the excretion of bile seemed almost continuous. Had the jaundice depended solely on the excretory function of the liver, the amount of bile secreted being the same, conditions must have been reversed. The importance of the secretion in this connection is further emphasized when we compare the columns of the table and note the gen-

TABLE 2.—RELATION OF JAUNDICE TO BILE EXCRETION (DUODENAL CATHETER)

(A) Unselected Cases

Case	Age, Days	Bile*	Jaundice	Food Supply	Remarks
1	5/8	—	0	Colostrum +	
	22/3	+	+	.....	
	32/3	0	++	.....	
	42/3	0	—	.....	
2	1/8	—	—	Colostrum +	
	11/8	—	+	Colostrum +	
	21/8	—	++	Colostrum ++	
	31/8	+	++	Milk +	Ten-minute test
	41/8	+	++	.....	Ten-minute test
3	1/4	—	—	Not fed.	
	11/4	—	+	Colostrum +	
	21/4	—	—	Colostrum ++	
	31/4	—	—	Colostrum ++	
	41/4	—	—	Milk	

TABLE 2—*Continued*

Case	Age, Days	Bile*	Jaundice	Food Supply	Remarks
	5¼	—	—	.....	Bile in stool
	6¼	+	—	.....	Ten-minute test
	7¼	—	—	.....	Castor-oil given shortly before test
	8¼	+	—	.....	Five-minute test
4	¼	—	—	Not fed	
	1¼	—	—	Colostrum —	Ten-minute test
	2¼	+	—	Milk	
5	⅛	—	—	Not fed	
	1⅛	—	+	Colostrum +	
	2⅛	—	++	Colostrum ++	
	3⅛	+	++	Milk	Bile in second test
	4⅛	+	++	.....	Bile in both tests
	6⅛	0	++	.....	
6	1	—	+	.....	Supplementary feedings begun at one hour
	2	—	—	.....	
	3	—	—	.....	
	4	+	—	.....	
7	¼	—	+?	Not fed.	
	1¼	—	+	Colostrum +	
	2¼	+	+	Milk	Bile in both tests
	3¼	+	++	.....	Bile obtained at once
8	⅛	—	—	.....	
	1⅛	—	+	.....	
	2⅛	+	±	.....	
9	1	+	+	.....	Bile in second test
	2	++	++	.....	Bile in first and second test
10	½	—	—	.....	Weight 6 lbs. 13 oz.
	1½	—	+	.....	Weight 6 lbs. 10 oz.
	3½	—	+++	.....	Weight 6 lbs. 9 oz.
	4½	—	+++	.....	Weight 6 lbs. 10 oz.
	5½	+	+++	.....	Weight 6 lbs. 11½ oz. Ten-minute test
	6½	+	++	.....	Weight 6 lbs. 13½ oz. Ten-minute test
11	11/12	—	+	.....	Early jaundice
12	1⅓	—	—	.....	Supplementary feeding begun at two hours
	3⅓	+	++	.....	Bile in stomach
	4⅓	+	+	.....	Ten-minute test
13	1¼	—	+	.....	
	2¼	—	0	.....	
	3¼	—	—	.....	
	4¼	+	—	.....	Bile obtained at once
	5¼	—	—	.....	

(B) Cases Slected on Account of Early or Marked Jaundice

14	3¾	+++	+++	.....	Bile in stomach
	4¾	0	++	.....	Bile in stomach
15	4½	+++	+++	.....	Bile in stoamch. Two-minute test
	5½	....	++	.....	Bile in stomach. Two-minute test
16	2	+++	+++	.....	A case of "Family Icterus." Infant saffron colored, skin dry, marked loss of weight, anuria. Bile in stomach at each test
	3	+++	+++	.....	
	4	++	+++	.....	
	5	++	+++	.....	
	6	+	++	.....	
17	6	+	+++	.....	Jaundice decreasing
18	3½	+	++	.....	
	4½	+	++	.....	Jaundice decreasing
19	5/6	+	++	.....	
20	3½	++	++	.....	Bile in stomach and vomitus
21	1½	++	++	.....	Bile in stomach and vomitus
22	3	++	++	.....	Bile in stomach
	4	++	+++	.....	Bile in stomach
	5	++	++	.....	
23	6¾	+	+++	.....	Bile in stomach
24	8½	+	+++	.....	Jaundice decreasing

* + = Present; — = absent; 0 = no examination.

eral parallelism of marked jaundice and marked bile. And yet the efficiency of excretion also plays a part, for it is clear that if excretion were adequate there would be no stagnation of bile, and the dilatation of the biliary capillaries, whilch is a definite and constant histologic accompaniment of icterus neonatorum, would not occur. It has, furthermore, been repeatedly shown by animal experimentation that jaundice cannot be effected, however greatly we may increase the secretion of bile, unless excretion is at the same time hampered. However, experiments of this nature on normal new-born animals would be of exceptional interest.

There are some other points noted in this table which require a word of explanation. In many of the cases a record was made of the time when colostrum appeared in the mother's breasts, of its profuseness, and of the day on which milk was first secreted. This was done with the purpose of ascertaining whether these facts bore any relationship to the occurence of jaundice. In addition, a number of infants, some of whom are included in this table, were given colostrum within the first few hours of life, expressed from the breasts of mothers whose children were 2 to 3 days old. The object of these tests was to determine whether, as has been claimed, starvation of the infant during the first day or two of life is an etiologic factor of the icterus. It was soon evident that it was a consideration of little moment; in the case showing no jaundice, the colostrum was noticeably scanty (Case 4), whereas in an instance in which colos-

trum feeding was begun when the baby was 2 hours old, jaundice was marked (Case 12). Furthermore, jaundice developed in a baby which gained 7 ounces during the first four days, owing to the fact that the mother's milk was profuse almost immediately after delivery. These incidents also show that the occurrence of jaundice but once in the series of fifty-two infants who had not been fed, was due not to the lack of food but to their time of life. The weights of a number of infants have been recorded, as a loss has been emphasized by many writers. It is not exceptional, however, for an infant with marked jaundice to lose but a few ounces and to regain its birth weight within the first week (Case 4). We have not considered critically the bearing of prematurity, of asphyxia, and of breach presentations in the etiology. It has been interesting as well as instructive to note how the predominance of jaundice in these abnormal conditions has been brought forward to strengthen the most divergent theories, from that of Morgagni, who laid the burden on the diminished pressure in the hepatic circulation, to those of the present day.

Summary

Tests by means of the duodenal catheter show that bile is very rarely excreted during the first twelve hours of life; it was obtained but once in the course of fifty-two tests.

Bile excretion during the subsequent twenty-four hours is variable; in cases of marked jaundice it is profuse; in cases not jaundiced it is scanty or absent.

The function of excretion gradually becomes fully established during the first week or ten days of life.

Where jaundice manifests itself, it precedes the excretion of bile into the duodenum.

Secretion of bile varies within wide limits. In general it is marked when the jaundice is marked.

The occurrence of jaundice results from a defective correlation of excretion and secretion. It is generally caused by the inability of the rudimentary excretion to cope with the sudden profuse secretion of bile.

Conclusions

Icterus neonatorum has always been the favorite domain of hypotheses, and happily I have no new theory to introduce into this overcrowded realm. It would seem beyond doubt that in most cases there is a disturbance of secretion, a polycholia, or according to Stadelman, a mere increase in the formation of bile pigment (pleiochylia). In many of the cases of marked jaundice this hypersecretion reaches an intesity so marked that bile overflows into the stomach, where it may be found on introducing the stomach tube, or manifests itself by its presence in the vomitus. The cause of this pleiochylia is not at present definitely proved, so that it would seem advisable in this instance to follow the principles of the physiology of bile secretion and assume, what seems probable, that it is due to an increased amount of available hemoglobin; that bile salts are absorbed

from the intestine, resulting in a disintegration of blood-cells and a consequent increased elaboration of bile in the liver. Whether or not this explanation of its histogenesis prove correct does not alter the fact of the usual occurrence of a hypersecretion.

In addition to the disturbance of secretion there is a coincident disturbance of excretion, and it is probable that were excretion as well provided for at birth as in later infancy, there would be no disturbance such as icterus neonatorum. We find, however, that the liver is most inadequately equipped to excrete this excess of bile; that during the first hours of life there is practically no functionating excretory mechanism, and that this function manifests itself but gradually. The bile is secreted under low pressure, but where an excess is secreted, as is generally the case in jaundice, the pressure is proportionately increased, so that a profuse excretion of bile into the duodenum is obtained. Nevertheless, congestion of the biliary capillaries ensues, as is shown by histologic investigations, and icterus results.

There has been too little consideration of the interrelation of secretion and excretion. The former may be said to be the more important, for in some cases in which there is but slight secretion, the rudimentary excretory function suffices to prevent the manifestation of icterus. That the lack of excretion cannot be the sole cause of the disturbance is evident when we consider that several cases of congenital obliteration of the bile ducts have been reported[17] in which icterus did not develop for days or even weeks, and yet this lesion may be considered comparable to the complete tying off of the common bile duct at birth. The complementary parts which secretion and excretion play in icterus neonatorum may well be compared to these functions in the breast after parturition. If the excretion of milk is copious and adequate, no pathologic phenomena result; however, if it prove inadequate, stasis ensues, lactose enters the blood stream and appears in the urine. The lactose is the counterpart of the bile. The reason why jaundice appears in the first days of life is because at the time when excretion has incompetely assumed its function throughout the body, in the liver as well as in other organs, for example, in the breasts and in the kidneys, a sudden flood of bile is poured into the passive excretory ducts and gains access to the hepatic circulation.

[17]Thomson, J.: Congenital Obliteration of the Bile Ducts, 1902.

THE PANCREATIC FERMENTS IN INFANTS

ALFRED F. HESS

PART I.—NORMAL AND CHRONIC CASES

For many years our lack of knowledge of the processes of intestinal digestion, and of the action of the glands which line the wall of the intestine, as well as those which pour their secretions into it, has hindered the study of the metabolic processes during infancy. It has frequently been regretted that we have to content ourselves with a direct study of the activities of the stomach, and cannot gain similar direct access for an investigation of the intestine, of the pancreas and of the liver. From time to time ingenious methods have been devised to overcome the inaccessibility of this region, and although most of these have proved of doubtful value, and hardly any have been satisfactorily applied to infants, it may be well to review them briefly.

The first methods were based on the principle of giving a food by mouth in such a form that it could not be digested by the stomach, and of subsequently examining the stool to judge of the effciency of the intestinal digestion. Sahli's glutoid capsule and Schmidt's nuclear tests based on this principle were devised to test the tryptic activity of the pancreas. However, as it has been shown that capsules are dissolved when given to dogs deprived of the pancreas,[1] and as Schmidt's test is also not reliable, the advisability of adapting these tests to our purposes may be dismissed.

A second method of investigation consists in examining the stool for the ferments themselves, especially for trypsin, but also for amylase, and for lipase or for the products of fat splitting. Many investigations have been carried out in this way to ascertain the secretion of trypsin. For this purpose the Müller-Schlecht[2] method, which depends on the digestion of serum, or the Volhard[3] and Gross[4] method, in which casein is substituted for serum, has been employed. A method of estimating the activity of intestinal diastase is also based on the above principles, and consists of a test introduced by Wohlgemuth[5] to judge of the starch-converting power of the stool. Similarly, but with less success, the stools have been examined for lipase, but more frequently for their content of free fat, fatty acids and soaps. The Cammidge reaction of the urine, said to be a test for glycerin, a product of fat-splitting, may be passed over with mere mention.

Still another principle is involved in the test of Boldyreff.[6] This consists in giving the patient oil by mouth, an oil-breakfast, which excites a regurgitation of pancreatic fluid into the stomach. The stomach con-

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[1]Frank, F.: Arch. f. Verdauungskr., 1912, XVIII.
[2]Schlecht, H.: München, med. Wchnschr., 1908, XIV, 725.
[3]Volhard, F.: München, med. Wchnschr., 1907, 403.
[4]Gross, O.: Arch. f. exper. Path. u. Pharmakol, 1908, LVIII, 157.
[5]Wohlgemuth, J.: Berl. klin. Wchnschr., 1910, p. 92.
[6]Boldyreff, W.: Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs., 1908, p. 209.

tents are evacuated and tested for the pancreatic ferments. We have seen but one report of a test by this method on an infant, and in that case it was not successful.[7] One of ehe difficulties is that the regurgitation from the duodenum does not always foilow. Moreover, in infants it would seem to be of doubtful value, on account of the necessity of putting a large amount of oil into the stomach.

One valuable source of information which has been grasped by those interested in this phase of physiology or pathology, is the occasional fistulæ of the pancreas or of the small intestine, which allow of the collection of their secretions. However, this exceptional opportunity, which has been taken advantage of from time to time since the famous observations of Beaumont in 1833, has never been accessible to those desirous of making a similar study on an infant.

The greatest advance in method has been made recently by the use of duodenal tubes. Almost simultaneously Gross[8] and Einhorn[9] published an account of a tube which when introduced into the stomach was carried by peristalsis into the upper intestine. Hemmeter[10] had attempted to use this direct method some years before. By means of this direct method, Einhorn and Rosenbloom[11] made a study of pancreatic ferments in the adult, which will be referred to below.

About two years ago a duodenal tube modelled after the Gross tube was devised for use on infants, and the early part of this study was carried out with its aid. It was found, however, that the method could be modified and greatly simplified for infants; that a simple catheter could be employed for these examinations. This duodenal catheter has been described in the pages of this journal in connection with the diagnosis and treatment of pylorospasm,[12] and in a study of icterus neonatorum.[13] It has also been briefly described in a paper on the bacteriology of the duodenum in infants,[14] and elsewhere,[15] so that it is unnecessary here to enter into the technic of its use. The catheter was used in this study of the pancreatic ferments because it seemed certain that an advance in our knowledge could be made by means of this method, which must be of greater value than the indirect methods which have preceded it. Just as it is preferable to test for rennet and for pepsin the stomach contents rather than in the stool, so in the case of the pancreatic ferments it is preferable to examine the duodenal contents rather than the stool. In the stool we have leukocytic ferments, bacterial ferments and other cellular ferments which possess proteolytic activity and are readily confused with trypsin. Schlecht's[2] report of the digestion of serum by means of a solution of old, thoroughly dried stool would even suggest activity other than ferment action, as a cause of proteolysis. In addition to the

[7]Ibrahim, J.: Verhandl. d. Deutsch. Naturf. u. Aerzte, 1908, p. 316.
[8]Gross, M.: New York Med. Jour., 1910, No. 2.
[9]Einhorn, M.: Med. Rec., New York, 1910, No. 3.
[10]Hemmeter: Complete reference not supplied.
[11]Einhorn, M., and Rosenbloom, J.: Arch. Int. Med., 1910, p. 667.
[12]Hess, A. F.: Amer. Jour. Dis. Child., 1912, p. 133.
[13]Hess, A. F.: Amer. Jour. Dis. Child., 1912, p. 304.
[14]Hess, A. F.: Jour. Infect. Dis., July, 1912, p. 71.
[15]Hess, A. F.: Arch. Int. Med., 1912, p. 37.

fact that the pancreatic ferments are mingled with non-pancreatic ferments in the feces—for besides these cellular enzymes, many others, for example, intestintal lipase, erepsin and probably intestinal amylase appear in the stool—the material for examination must naturally be contaminated with unutilized food products. But of more weight is the fact that we have no means whatsoever of even estimating how much ferment has been absorbed or utilized in the small intestine. That this factor is large we can infer from our knowledge that this part of the intestine is the seat of the physiologic activity of these ferments, and that they must be largely used up in this process. Experiment confirms us in this opinion, for it has been demonstrated that the ferments of the pancreas are hardly present in chyme obtained from a fistula in the cecal end of the ileum.[2] Realizing this defect in method, and in order to allow as little time as possible for absorption, a cathartic is given before obtaining stools for the ferment tests.

Such are the criticisms which justly can be levelled at any tests of stool for pancreatic ferments, but which a direct duodenal test is not exposed to. However, the direct method which we have used in this study, like most methods, is likewise not perfect. It may truly be argued that the duodenal fluid may suffer an admixture of gastric juice. At present this cannot be obviated; however, if all duodenal fluid which is acid is discarded, and only alkaline or neutral juice examined, this objection loses much of its force. We should realize also that the pancreatic juice obtained in this way cannot be considered pure, as it contains bile as well as some succus entericus. These criticisms would apply forcibly were we pursuing a purely chemical study of pancreatic enzymes. However, we have not undertaken a study of this nature, but rather an investigation of the activity of duodenal digestion, in which the pancreatic ferments take a pre-eminent part, and the bile and other juices play a minor but by no means negligible rôle. Moreover, there is no other way in which a pancreatic juice even comparatively pure can be obtained.

When we inquire into the present status of our knowledge in regard to the activity of the secretions of these glands in infancy, we find that one of the most modern and exhaustive books on the physiology of the digestion of the infant, that of Czerny and Keller,[16] confines itself largely to a discussion of the results of post-mortem investigations, "in view of the fact that the secretions of the intestinal glands and the functions of these secretions are not accessible to direct examination *intra vitam*." Tests of this nature, made with the watery extract of a gland, have a very limited application to the physiologic activity of an organ, and can be considered of only temporary value in lieu of something better. This becomes all the more evident when we reflect that merely the proferment may exist in the gland parenchyma, and that an activating substance, such as enterokinase in the case of trypsinogen, may be necessary to render the ferment potent. We shall have occasion to refer to these investigations based on post-mortem material, and also to those undertaken by means of the examination of the stool. There has been no direct test of the pancreatic ferments of infants.

[16]Czerny, A., and Keller, A.: Des Kindes Ernährung, etc., 1906, I, 66.

Technic of Chemical Tests

The fluid was brought to the laboratory as soon as possible, generally within an hour or two and subjected to the following tests:

Amylase Test: One c. c. of duodenal fluid was added to a tube containing 10 c.c. of freshly prepared 1 per cent. starch solution, and 1 c.c. of toluol. A control tube contained only starch solution and toluol. Both were placed in the thermostat at 37 C. for forty-eight hours. The mixtures were then made up to 50 c.c. by the addition of water, and run from a burette into 10 c.c. of Fehling's solution ((Fehling's solution equals 5 c.c. of the alkaline solution, plus 5 c.c. of the copper solution, plus 30 c.c. water). Ten c.c. of this solution represents 0.002 gm. of glucose. As an indicator of the complete reduction of the Fehling's solution concentrated potassium ferrocyanid (2 to 3 c.c.) and glacial acetic acid (a few drops) were used.

Lipase Test: One c.c. of the duodenal fluid was added to 1 c.c. of neutral ethyl butyrate, 1 c.c. toluol, 10 c.c. water. As controls a test of the duodenal fluid and one of the ethyl butyrate were made. The three tubes were incubated for twenty-four hours, and titrated against N/20 NaOH, using phenolphthalein as an indicator. The acidity of the duodenal fluid, and of the butyrate solution, was then deducted to obtain the net amount of acid due to lipolytic action.

Trypsin Test: In the early tests made over a year ago Mett's tubes were used. As is well known, these consist of tubes of small caliber containing coagulated egg-white. These were found serviceable for qualitative tests, but unreliable for quantitative estimations. It was found that tubes of 5 per cent. gelatin were fully as reliable and much more practicable, so that this method was followed in most of the tests. To a tube of gelatin 0.5 c.c. of duodenal fluid, 0.5 c.c. of toluol, as well as 0.25 c.c. of N/1 NaOH were added. The control consisted of a tube containing gelatin, sodium hydrate, and toluol. Tubes prepared in this way were incubated generally for twenty-four and for forty-eight hours, and then placed in the refrigerator. The time necessary for liquefaction was noted, and also whether this change was complete or incomplete. Casein was not used in these tests as it is well known that it is reacted on by erepsin as well as by trypsin.

In addition to these chemical tests the amount, the color, the consistency and the reaction of the fluid to litmus and to phenolphthalein were noted. At the time of the passing of the catheter, the amount of the stomach content, its reaction to litmus and to Congo paper, were recorded, as well as various secretory and motor phenomena.

Scope of Original Investigation

The following direct study of pancreatic ferments by means of the duodenal fluid may be grouped thus:

1. Normal cases:
    a. Tests before the infant had nursed (during first half day of life).
    b. Tests during first week of life.
    c. Test on older infants (one week to one year).

2. Pathological cases:
   a. Tests in cases of marasmus (atrophy, decomposition).
   b. Tests in cases of pylorospasm associated with gastric hypersecretion.
3. Chemical and physiologic observations on the duodenal secretion.

1. *Normal Cases.—a. Tests of Unfed New-Born Infants.*—The first tests were carried out on infants varying in age from a few weeks to a few months, but it soon became evident that in order to get a comprehensive view of the subject, the ferments ought to be sought from the day of birth, even before they had been put to the breast. Accordingly, a large number of infants were examined by means of the catheter during the first few hours of life, to ascertain whether the pancreatic ferments are present in the duodenum at this period and whether they are secreted independently of the stimulus of colostrum. The passage of the catheter in the newborn is very easily accomplished, as has been recently pointed out in an article on icterus neonatorum. The two investigations were pursued simultaneously; that is to say, the fluid which was aspirated was considered both in regard to the presence of bile and the pancreatic ferments. The examination was by no means always successful, for although access to the duodenum was almost always possible, frequently the upper intestine was empty and no fluid could be aspirated. In a few instances, in these very young infants, a slight admixture of blood rendered the fluid valueless for our purposes; and in some other cases the scanty duodenal contents were rendered acid by an admixture of gastric juice. For these various reasons, although fifty-five tests of the "unfed new-born" were attempted, duodenal juice was obtained in only eleven, and in no instance in sufficient quantity to make tests for all three ferments, for trypsin, lipase and amylase. However, a test for one ferment was made in one case, and for another in the next, using all the ferment that was secured, with the object of ascertaining whether these pancreatic ferments are present in the intestine before the ingestion of any food.

Before proceeding to detail the results, it will be of advantage to review the previous work in this field; and as the investigations on this group of infants, those who had not nursed, are so few in number, it would seem advisable at the same time to include reports of infants who were somewhat older. The greatest divergence of opinion has always existed as to the time of appearance of amylase. Zweifel,[17] who made use of extracts of the pancreas, failed to demonstrate the ferment in infants under two months of age, and Korowin[18] confirmed these results. Moro,[19] however, repeating this investigation, but making use of an improved technic, more especially by extracting the pancreas for a longer period and at a higher temperature, demonstrated the presence of diastase in the pancreas of an infant which died at birth, and of some others which had lived for a few days. Ibrahim[7] found amylase in the pancreas of the new-born and of the premature infant, and numerous ferments in the meconium.

[17]Zweifel: Untersuch. u. d. Verdauungsapparat d. Neugeb., 1874, p. 35.
[18]Korowin, J.: Jahrb. f. Kinderh., 1875, VIII.
[19]Moro, E.: Jahrb. f. Kinderh., 1898, III, 342.

As is well known, trypsin is not present in the pancreas, but exists in the form of its proferment, trypsinogen, which is activated by the enterokinase of the intestine. Both trypsinogen and enterokinase have been found in the fetus, and trypsin in the stools during the first day of life.[20] Another proteolytic ferment, erepsin, which we shall have occasion to refer to later, has likewise been found in the fetus.[21]

Lipase has been demonstrated in the meconium in small amounts during the first day of life,[20] and, according to Sedgwick,[22] a gastric lipase of considerable lipolytic power may be obtained from the stomach by the second week.

It will be noted that most of these examinations have been made either on post-mortem material, or on infants somewhat older than those comprising our first group. In our experience trypsin and lipase were found most constantly. The former in nine out of eleven tests, and by no means as a mere trace, but sufficient frequently to digest the gelatin in twenty-four to thirty-six hours. Lipase was demonstrated in four of the five tests, also in considerable quantity—from 0.5 c.c. to 2.9 c.c.—as measured by titration with 1/20 N. NaOH solution. Lipase of uncertain origin was found in small amount in the stomach contents. Amylase was present twice and absent six times. This positive reaction was not due to an admixture of saliva, for no saliva was present in the material examined; the one case belonged to a series of four, in which a dilute solution of alum was swabbed on the papillæ of the salivary ducts, resulting in complete inhibition of salivary excretion. From these tests we conclude that the three pancreatic ferments are frequently present in the duodenum at or soon after birth. The youngest infant in which amylase was demonstrated was one and a half hours old.

The results of these tests, comprising a narrow field, are of physiologic rather than of clinical interest, and need no clinical commentary. The accepted mechanism of pancreatic secretion is that secretion is elaborated by the mucosa of the small intestine, enters the portal circulation and acts as a hormone to stimulate the pancreas. There is no way of judging whether secretin, in our cases, acted as the messenger to stimulate the pancreatic activity; it has been found in infants which died at birth, but it is not at all times present in the intestine of the new-born. Whatever the mechanism, it is clear that food is not needed to stimulate the activity of the pancreas. The hydrochloric acid which is regularly present in the stomach at birth, as we shall show at another time, would seem to be sufficient to initiate the excretion of pancreatic juice into the duodenum.

b. Tests During the First Week of Life.—Fifty tests were made on thirty-three infants varying in age from one-half to seven days, the same method of examination being employed as in the cases of the former group. Some of these tests did not include an analysis of the duodenal contents, but were made for the purpose of obtaining gastric juice or bile, of determining the state of the pyloric sphincter, or to obtain material

[20]Hecht, A.: Die Faeces d. Säuglings, 1910, p. 153.
[21]Langstein, M. and Soldin, M.: Jahrb. f. Kinderh., 1908, p. 9.
[22]Sedgwick, J.: Jahrb. f. Kinderh., 1906, p. 194.

for bacteriologic investigation. However, in forty-one instances one or more of the three pancreatic ferments were investigated. As was to be presupposed from our experience with the younger infants, the presence of all three ferments was demonstrated. Trypsin was regularly found in quantity sufficient to digest 5 per cent. gelatin within twenty-four hours; in only two of nineteen tests was it absent. Through an error, 10 per cent. gelatin was used for several of these tests, and as gelatin in this concentration is not applicable as a test for trypsin, these examinations were necessarily discarded.

Lipase was found in all of the ten fluids in which it was tested. In infants one week of age the duodenal juice obtained within an hour is insufficient in amount to carry out the tests with each fluid, for 2 c.c. are necessary for the lipase test, one for the amylase test, and 1.0 c.c. for trypsin. If only a small amount of fluid was obtained, trypsin was tested for, but unless the fluid was neutral or alkaline to litmus, it was not examined for ferments. The lipase varied in quantity from 0.25 c.c. to 2.4 c.c., almost all being under 2.0, as judged by titration with 1/20 N. NaOH.[23] It is not possible to ascribe this variation solely to a difference in the quantity of lipase secreted, as the fluids examined were not sufficiently pure to allow of this deduction. In addition to a difference in the amount of lipase, there are numerous factors which may well have contributed to these varying results. For instance, the dilution of the pancreatic lipase with succus entericus or with gastric juice; the presence of more or less bile, which would tend to dilute the pancreatic secretion, although at the same time augmenting its lipolytic action; the variation in the reaction of the juices. Experiments undertaken at the outset of this work clearly demonstrated that a slight addition of Na_2CO_3 or of NaOH to the fluid was sufficient to alter its ferment action. In view of these facts it seems advisable, for the present at least, to consider these tests from a qualitative rather than from a quantitive point of view. Nevertheless the difference in lipase is so great that we seem justified in concluding that in infants of this age there is a marked normal variation in its activity.

Amylase was tested for in nineteen cases and found in every instance. It was present in larger quantity than in the cases of group *a*, and in several instances completely reduced the starch solution. Some cases could not be tested for amylase on account of the admixture of saliva to the fluid. However, that saliva did not account for the amylolytic reaction is proved by the fact that in numerous instances the stomach contents failed to reduce starch, whereas the duodenal fluid possessed this activity, and by two instances in which the secretion of saliva was once more inhibited by a local application of alum.

c. Tests on Older Infants.—The group of older infants comprises twenty tests carried out on fourteen infants, varying in age from two weeks to one year. As we had already determined that all the ferments are present in very young infants, these examinations were studied with

[23] In giving figures of the amounts of the ferments we realize that they do not represent a quantitative estimation of the ferments but merely denote its activity.

the purpose of ascertaining possible quantitative differences in the amount of juice obtained, or in the potency of the ferments, compared to that of infants during the first week of life, or of defining a relationship between the composition of the food and the secretion of the three ferments. These infants were of about normal weight; we could not, however, be certain in many cases that they had not at a previous time suffered from intestinal disturbances. Two were nursing babies, the others fed in most instances with a mixture of milk, containing approximately 2 per cent. fat, 6 per cent. sugar and 2 per cent. protein. This group should have included a large number of healthy breast-fed babies, but it was not possible for us to obtain many normal nurslings for this examination, so that an extension of this test had to be deferred. As was to be expected, a far greater quantity of duodenal juice was obtained in this group of cases. However, although the difference in this respect was marked between infants of one week and one month, it was slight between those of a month or two and those of five or six months. It should be borne in mind that the total secretion for twenty-four hours was never collected, as this was not practicable.[24]

It was found that whereas infants during the first week of life secreted about 2 c.c. of duodenal fluid in the course of a test of a half hour, infants a month or two old secreted 4 to 5 c.c. On surveying the results of the tests of infants of this group, it seems as if we can make only broad quantitative deductions. Trypsin, it will be remembered, was present to a marked degree in younger infants, as was lipase, and it is only in the case of amylase that we can state that an enzyme was present in older infants in increased amount. For, whereas in the cases under group *b* the glucose formed by the amylase was never sufficient to entirely reduce the 10 c.c. of Fehling's solution used for the test, in these older infants, frequently half of the incubated fluid was able to effect this result. The most marked amylolytic action was obtained with the fluid from an eleven-weeks-old breast-fed infant, in which case 12.7 c.c., that is, about a quarter, of the test fluid completely reduced the Fehling's solution. These results have a clinical bearing which will be discussed below.

It has been asserted by some,[25] but denied by others, that the pancreas has a selective activity; that when the food contains a great deal of fat, for example, a lipase of proportionately marked lipolytic power is secreted, and so with the other ferments. Judging from our experiments this would not seem to be the case, for a baby fed on skimmed milk had a lipase equivalent of 3.0 c.c., two fed on milk with 3 per cent. fat had a lipase titrating at 2.2 c.c. and at 2.4 c.c., and still others being fed on milk containing 2 per cent. fat had a lipase varying from 0.9 c.c. to 6 c.c. Nor could a parallelism in this respect be drawn from the results of the protein and starch content of the food, and the respective amounts of trypsin or amylase in the duodenal fluid. Indeed, if the relationship exists, we should expect the nursing infant to have no amylase whatso-

[24]Recently we have devised a catheter which is self-retaining and with its aid we hope to be able to overcome this difficulty and to collect the secretion for long periods.

[25]Pavlov, I.: The Work of the Digestive Glands, Ed. 2, 1910, p. 140.

ever, or at least a marked deficiency compared to an infant receiving a decoction of cereal. On the contrary, the amylase of the two nursing babies was quite as active as that of infants taking milk and barley water, which, from a clinical point of view, would argue strongly against absolute specificity in the secretion of the pancreatic cells.

2. *Pathologic Cases.—a. Marantic Infants (Atrophy, Decomposition).*—It was with especial interest that we tested a number of infants suffering with severe marasmus, for a lack of intestinal ferments, the more particularly as a deficiency of pancreatic ferments has been suggested as the cause of this ailment. A few years ago Wentworth[26] presented the hypothesis that marasmus was due primarily to the fact that the infant received an improper food, resulting in the secretion of an inadequate gastric juice, which in turn failed to stimulate the cells of the duodenum to elaborate normal secretin. As the flow of pancreatic juice is supposed to depend for stimulation on this hormone, an insufficiency of ferments naturally results. This hypothesis was based on finding a diminished amount of secretin in the mucosa of the intestine of the atrophic, compared to the normal, infant.

Unhappily there is little difficulty in finding marantic infants in hospitals; fourteen were tested. many were greatly wasted, most of them weighed 6 to 7 pounds, although 2 to 6 months old. It was soon evident that these infants had pancreatic juice containing enzymes of no mean activity. The lipase frequently titrated at 2 to 3 c.c., the amylase converted sufficient starch solution to completely reduce the Fehling's solution, and the trypsin digested the gelatin within twenty-four hours. This was not the exception, but rather the rule. Nor can we fall back on the assumption that the total quantity of pancreatic juice was deficient, for it was fully as copious as in the former group of cases. Indeed in marked marasmus we occasionally meet with a most interesting form of pancreatic hypersecretion, quite different from the cases associated with gastric hypersecretion which will be considered below, a form which may be termed "paralytic hypersecretion." In these cases, there is an almost continuous flow of alkaline duodenal fluid, containing all three pancreatic ferments. The following two instances are typical:

Case 1.—W. E., 3 months old. Marasmus. Weighed 6¾ pounds. Receives 2 ounces of milk every two and one-half hours containing approximately 2 per cent. fat, 6 per cent. sugar, 1 per cent. protein.

October 30, 1911. Catheter passed one and one-half hours after feeding; stomach almost empty; contains a small amount of neutral fluid. Pylorus readily traversed. In three-fourths hour 12 c.c. of fluid was aspirated; in one hour 20 c.c. This fluid was obtained while the end of the catheter was about 5 cm. below the pylorus. It was yellow, viscid, alkaline. The secretion was noted as the most constant and profuse yet obtained. When the test was discontinued after one and one-half hours, the secretion seemed somewhat less marked. At this time a test of the stomach contents, which were very scanty, gave a neutral reaction. The

[26]Wentworth, A.: Jour. Am. Med. Assn., July 20, 1907, p. 204.

infant died four days later. This fluid was 0.2 acid to 1/20 N. NaOH. It contained no lipase, amylase 50, and digested gelatin within twelve hours.

Case 2.—December 11, 1911. J. C. 5½ months old. Weight 6⅔ pounds. Fed every three hours, 4 ounces of a mixture containing 13 ounces whole milk, 11 ounces water, 1 ounce dextrin malt preparation. Test two and one-half hours after feeding.

2:25, about 2 ounces of food evacuated from stomach; gas, foul odor.
2:30, catheter passed into duodenum.
2:40, 10 c.c. of alkaline, somewhat cloudy, yellow fluid aspirated.
2:55, 8 c.c. more fluid obtained.
3:00, 6 c.c. watery, neutral yellow fluid.
3:07, 3 c.c. slightly alkaline, faintly bile-colored fluid.
3:12, 2 c.c. darker, thicker, more alkaline fluid.
3:18, 3 c.c. neutral fluid.
3:27, 6 c.c. yellow, slightly alkaline fluid.
3:35, 2 c.c. alkaline, cloudy fluid.
3:40, 2 c.c. more fluid.
3:45, catheter withdrawn. In seventy-five minutes 45 to 50 c.c. of duodenal fluid was aspirated. The amount of secretion in this wasted infant equaled that of the normal adult.

A previous test three days before had shown a similar but somewhat less marked secretion. At that time the fluid tested as folows: 0.3 acid to 1/20 N. NaOH, lipase 0.9 c.c., amylase 33.5 c.c., gelatin digested in twenty-four hours. The fluid obtained at the second test was 0.1 acid, gave a reaction of 0.7 c.c. to lipase, of 29.3 c.c. for amylase, and digested the gelatin in twenty-four hours.

Here are two instances of a hypersecretion of pancreatic juice in markedly marantic infants, where the normal stimulus of hydrochloric acid was absent or greatly diminished. We have termed this condition paralytic succorrhea or hypersecretion because it seemed to resemble the paralytic secretion of the salivary glands which Claude Bernard[27] was able to incite by dividing the chorda tympani nerve. In dogs an anomaly of secretion similar to that which we have described has been observed, as the result of a diseased condition of the pancreas—continuous watery pancreatic secretion similar to a transudate.[28] In these animals changes denoting exhaustion were found in the glandular acini; it would be interesting to compare this condition with the microscopic picture of the pancreas in a case of "paralytic hypersecretion" in an infant.

b. Pylorospasm Associated with Gastric Hypersecretion.—We have made a separate grouping of the cases of pylorospasm because generally they are associated with an abnormality of secretion, the secretion of gastric juice. Clinically they are frequently included in the general classification of marasmus, a name used to cover a great variety of conditions. However, in most marantic infants hydrochloric acid is deficient, whereas in these cases it is increased, and we have a status which closely

[27]Luciani, L.: Physiologie d. Menschen, 1906, II, p. 62.
[28]Luciani, L.: Physiologie d. Menschen, 1906, II, 75.

resembles simple starvation, brought about by an insufficient quantity of food entering the intestine. In the ordinary case of marasmus, on the contrary, enough food reaches the intestine, but is not utilized as there is some disturbance of absorption or of assimilation. So that from a metabolic point of view the two groups of cases are quite different, although they are characterized by the same chronic atrophic condition. As might be expected, the increased secretion of hydrochloric acid stimulates an increased flow of pancreatic juice, and we find a duodenal or pancreatic succorrhea. This may be termed "functional pancreatic hypersecretion." A study of the duodenal juice of seven cases of this type shows that the secretion is rarely as profuse as in paralytic hypersecretion, and that it differs from these markedly in character. It is not thin and watery, but contains an average amount of lipase and other ferments, and is exceptional merely in being profuse. In one instance it was clear that for some time a hypersecretion of gastric juice stimulated a hypersecretion of pancreatic juice; this stage was followed by one characterized by a marked diminution of gastric secretion, and "functional pancreatic hypersecretion' gave way to a succorrhea of the paralytic type.

3. *Chemical and Physiological Observations on the Duodenal Secretion.*—In the course of the many tests in the clinic and in the laboratory, we had opportunity to make interesting observations concerning the secretion of pancreatic juice and the properties of the juice itself. It was found that the pancreatic juice generally began to be excreted a few minutes after milk was given, and that it was likewise stimulated by introducing water or a 0.5 per cent. hydrochloric acid solution into the duodenum. These methods were not frequently resorted to in order to obtain the juice as they vitiated the tests. The examinations were almost always undertaken two to three hours after feeding for earlier tests introduced the error of food contamination, and later tests were found to yield a scanty amount of fluid. The mechanical irritation of the duodenum had no effect in inducing pancreatic secretion, nor was secretion stimulated by the infants sucking the catheter or a nipple. Generally pancreatic juice and bile are excreted simultaneously, the amount of bile varying, so that the color of the fluid may be a light or a golden yellow. At times in some cases, and repeatedly in others, pancreatic juice was obtained free from bile; in one case in the course of several tests bile was obtained only once. The duodenal fluid was neutral, alkaline or acid; when acid it was not saved for examination. It is commonly stated that the reaction of the duodenum is alkaline; this, however, was not found to be the case, for the reaction depends entirely on the resulant of the interaction of the alkaline pancreatic and intestinal fluids, with the acid gastric secretion. They may neutralize each other; there is frequently an excess of acid; the reaction is rarely alkaline except in the fasting period. As has been noted in animals, the fluid increases in alkalinity as it flows. When the juice is acid it is turbid, when alkaline, or when rendered alkaline by means of NaOH, it becomes clear. It was noted also that a slight degree of acidity did not markedly inhibit the action of lipase, but that an increase in alkalinity did retard its enzyme action.

In the case of trypsin, on the contrary, a slight acid reaction of the fluid almost nullified the proteolytic activity.

Conclusions (Part I)

Whatever progress this investigation is able to offer is due to the introduction of a new method. Heretofore we have had to content ourselves with a study of the pancreas after death or of the stools during life, to gain an insight into the activity of the pancreatic ferments. With the aid of the duodenal catheter, which was devised for the diagnosis and treatment of pyloric obstruction, the opportunity presents itself for the first time of making a direct test of the duodenum in infancy, a test similar to that which is employed in investigating the secretions of the stomach.

By this means fluid was aspirated from infants varying in age from a few hours to a year, some normal, some ailing and was examined for amylase, lipase and for trypsin.

A considerable number of new-born infants were tested before they had been put to the breast. It is of physiologic rather than of clinical interest to note that without the stimulus of food to incite secretion, the three pancreatic ferments were found in the intestine.

During the first week of life the amount of the pancreatic secretion is still very scanty, but it contains the starch splitting enzyme with increased regularity.

In older infants, a month or more of age, there is both an increase in the quantity of pancreatic juice, and a decided augmentation of its amylolytic power. This is a most remarkable functional activity, and one difficult of biologic explanation; for to what purpose is the starch-splitting ferment adapted at this age? It would seem as if the infant was furnished with enzymes competent to digest not only its natural food, mother's milk, but also artificial food. This amylase has the power of digesting a 1 per cent. starch solution, or, for example, barley water, and therefore bears out the old contention of Jacobi[29] and of Heubner[30] that food containing starch can be utilized by the infant in the first month of life.

It seems unwise at present to draw conclusions of an absolute quantitative nature from the tests of ferments; however, we were unable to demonstrate a selective activity of the pancreatic juice—such as an increased strength of amylase when barley water was mixed with the milk, or of lipase when the percentage of fat was increased. The secretion of a large amount of amylase by the nursing baby would also argue against absolute specificity in this regard.

Some sick infants were tested. It was soon evident, in a study of marasmus, that the hypothesis is untenable, which suggests that this disease is associated with or is caused by a deficiency of these ferments. Even in advanced cases they were not lacking. It would seem probable, therefore, that absorption is not essentially defective, for the pancreatic enzymes are concerned mainly with the preparation of food for absorp-

[29] Jacobi, A.: Gerhardt's Handbuch, etc., 1876, I.
[30] Heubner, O.: Berl. klin. Wchnschr., 1895, p. 201.

tion, but that the essence of this disturbance is one of retention and assimilation.

Some of these atrophic infants, although secreting little gastric juice to act as a stimulus, secreted a very large amount of thin, watery juice, containing all the pancreatic ferments, although weak in lipase. This pathologic condition we have termed "paralytic hypersecretion or succorrhea," as it suggested the hypersecretion following the severing of the chorda tympani nerve. The loss of alkali due to the outpouring of this secretion, which serves no end, as it does not neutralize acid, may constitute an important loss to the body, and seems worthy of further study. This is all the more probable when we consider that dogs with pancreatic fistulæ cannot be kept alive for long periods unless they are given alkali.

Cases of pylorospasm associated with gastric hypersecretion furnished numerous examples of pancreatic overactivity of a quite different nature. In these instances the gland probably was overstimulated by the normal agent, the gastric juice. Such cases may be termed "functional pancreatic hypersecretion," and later may develop into the paralytic type.

It gives me pleasure to acknowledge my indebtedness to Dr. Chapin of the Post Graduate Hospital, for the privilege accorded me of carrying out a large part of this work in his wards, and to thank Miss Mildred Fish and Miss Gertrude Fisher for their able assistance.

Part II. In Acute Intestinal Indigestion (Alimentary Intoxication)

The large series of cases of part one does not include any examination made on infants suffering from *acute* disturbances. Although numerous tests of this nature had been carried out, they were deemed at the time insufficient in number to be classed as a group, and were therefore not brought forward for consideration. Since then, however, other cases of this description have been tested, almost all suffering from acute intestinal indigestion of moderate or severe grade. From the first beginning of catheterization of the duodenum, which now dates back over two years, two groups of sick infants seemed to offer the greatest promise for experimental investigation of the pancreatic ferments, that of malnutrition which has been already considered, and another disease which is likewise characteristic of infancy and one of the largest factors in infant mortality, variously termed acute intestinal indigestion or alimentary intoxication.

In this further paper on this subject we report on examinations of cases of the second group, acute intestinal disturbances. The number of cases which form the basis of the present study is not large, but as the seasonal incidence of the disease would necessitate our again waiting for a long period before more cases presented themselves, it seemed advisable to publish some interesting data on this important topic.

The technic employed was in all respects the same as in the previous investigation of pancreatic ferments. For the trypsin test, gelatin and egg albumin were used, for amylase a 1 per cent. starch solution, and for lipase neutral ethyl butyrate. We realize that these methods do not furnish quantitative tests for these three ferments, but afford merely an

index of ferment activity. It is realized, likewise, that all quantitative estimations of ferments are fraught with error. In view of this inherent deficiency of our present methods, the subject was regarded in the previous study almost entirely from a qualitative point of view. However, the estimations to be reported, when considered collectively, vary so widely from those obtained on normal infants, or indeed from those suffering from chronic malnutrition, that a comparison on the basis of quantitative results seems justified.

Thirteen acute febrile cases were tested by means of the catheter, comprising a total of eighteen tests. These infants varied in age from six weeks to nine months. Of the thirteen, all but three were cases of acute intestinal indigestion (alimentary intoxication). The three non-intestinal cases included pneumonia, empyema and adenitis, and are considered with the others to advance the question whether the results which we obtained are attributable to the acute intestinal disturbance or are common to all febrile conditions.

For purposes of comparison, we have framed our results in a table (Table 1), which affords a clearer picture than mere description. The tabulated cases have been divided into four groups: (a) Cases of acute intestinal indigestion, (b) other fabrile cases, and two groups of chronic disturbances, (c) marasmus, and (d) marasmus accompanied by pancreatic succorrhea. In all groups, it is seen that tryptic activity was adequate; this ferment has always been found the most constant. When we review our results of amylolytic activity, although we find noticeable differences in the figures obtained, they are not sufficiently marked or constant to draw distinctions.[31] It is different, however, in regard to lipase. In almost all cases of acute intestinal disturbances the lipase was found to be deficient, in some cases almost absent. It should not be thought that this was merely a premortal phenomenon, for, although most of the infants were suffering from severe disturbances all but three recovered. The cases of marasmus which we record (c) were in a far more critical condition than these acute cases. Their respective ages and weights are appended to show the extreme degree of their atrophic condition. Nevertheless, all the pancreatic ferments, including lipase, were generally present in normal amount. In the acute intestinal cases the deficiency of lipase seemed to bear to some degree a direct relation to the severity of the illness. In the mildest case which we tested, the lipolytic action was merely somewhat reduced. Furthermore, in a case showing the classical symptoms of alimentary intoxication (No. 7), including the stupor and glycosuria, in which we carried out three tests, we obtained far larger amounts in the early stage of the illness and during the period of beginning recovery than in the test made during the height of the illness.

[31]It should be remembered in this connection that the higher figures denote a smaller and not a larger amount of amylase, as they refer to the volume of starch solution (acted on for forty-eight hours by the amylase), which it was necessary to add in order to completely reduce the Fehling solution. The reverse is true as regards the figures for lipase; they bear a direct relation to the amount of fatty acid formed during the incubation of the neutral ethyl butyrate with the ferment. The details of these tests are given in the previous paper.

TABLE 1.—TESTS OF PANCREATIC FERMENTS

A. Cases of Acute Intestinal Indigestion (Alimentary Intoxication)

Cases	Age Months	Lipase	Amylase	Trypsin	Remarks
1	2	1.7	30.6	24 Hours	.....
2a	....	0.9	....	.....	.....
b	3	0.7	50.0	24 Hours	Death 48 hours after second test.
3	2½	3.2	46.5	24 Hours	Mild case.
4	3½	1.8	50.0	48 Hours	.....
5a	....	1.3	42.0	24 Hours	.....
b	6	1.0	48.5	.....	.....
6	1½	0.3	50.0	24 Hours	Death after 24 hours.
7a	....	1.4	50.0	24 Hours	.....
b	4	0.1	....	24 Hours	.....
c	....	1.6	41.0	.....	.....
8	9	1.4	50.0	48 Hours	.....
9a	5	1.2	39.5	24 Hours	.....
b	....	1.6	48.0	.....	.....
10	4	0.4	50.0	24 Hours	Death after 48 hours.

B. Other Febrile Cases

11	4	3.3	....	.....	Pneumonia.
12a	....	2.4	39.5	.....	.....
b	6	4.2	48.0	.....	Empyema.
13	4½	5.1	43.5	24 Hours	Cervical adenitis.

C. Marasmus

14	6	3.0	31.0	24 Hours	Weight, 6 pounds, 15 ounces.
15	16	8.7	17.3	Digested Egg White	Weight, 9 pounds, 3 ounces.
16	4	3.8	30.3	Digested Egg White	Weight, 6 pounds, 8 ounces.
17	2	4.8	25.0	.....	Weight, 6 pounds, 8 ounces.
18	2½	3.4	50.0	24 Hours	Weight, 6 pounds, 2 ounces.

D. Marasmus Accompanied by Pancreatic Succorrhea

19	2½	.0	50.0	24 Hours	A second test also showed absence of lipase.
20	2	7.8	50.0	24 Hours	20 c.c. of golden alkaline fluid aspirated in 50 minutes.
21	5½	0.9	33.5	48 Hours	45 c c. of yellow alkaline fluid aspirated in 75 minutes.
	....	0.9	29.3	24 Hours	

It seems unnecessary to give in detail the clinical history of the various cases; however, in order to give an idea of the type of case referred to, we shall summarize the clinical history of a few:

CASE 1.—M. C., aged 2 months, entered the hospital with a history of five days illness accompanied by fever, slight vomiting and marked diarrhea; 6 to 10 watery stools a day. Child was moderately nourished; weight 9 pounds 8 ounces. Prostrated. Typical appearance of cholera infantum. Treatment *Eiweissmilch*. Recovery.

CASE 2.—Aged 3 months. Very similar to the previous case. Death forty-eight hours subsequent to examination.

Case 3.—Aged 2½ months, suffering for about ten days from enteritis; six to seven stools a day, containing undigested food.

Case 4.—Aged 3½ months; weight 9 pounds 4 ounces. Well until forty-eight hours previously. Acute onset with vomiting and diarrhea. Temperature between 103 and 104 F. Moderate diarrhea; no vomiting in hospital.

The question naturally suggests itself as to whether the deficiency of ferment secretion may not be dependent merely on the febrile condition. With this doubt in mind some non-intestinal febrile cases were examined. In a test made on an infant suffering from pneumonia and recording a temperature of 104.3 F., in another admitted to the hospital for empyema with a temperature of about 103 F., and in a third which had cervical adenitis and a temperature ranging for some days between 101 and 104 F., it will be seen (Table 1 B) that lipase was not found deficient. It would seem as if there were something distinctive in the relation of the lipase deficiency and the intestinal disturbance. There is a group of cases which was described in the former paper under the designation of "pancreatic succorrhea," in which a low lipolytic activity was frequently found (Table 1 D). In these cases, which have been encountered among atrophic infants, there is a marked hypersecretion of watery pancreatic juice, which is especially weak in lipase. With the exception of this group in which the lack of lipolytic activity must in a large measure be offset by the increased quantity of pancreatic fluid secreted, we have not met with instances manifesting this deficiency.

Discussion

It would be unwise to draw broad deductions from these tests, as further examination may show that in some instances lipase is not lacking. However, in view of the fact that this ferment alone of the three pancreatic enzymes, was found lacking in acute intestinal disturbances, it may be in place to add a word as to some closely related clinical and metabolic manifestations of this disorder. Clinicians are agreed that infants suffering from acute intestinal derangements tolerate fat poorly, and indeed only slowly regain the power to assimilate this food-stuff. We do not wish to insist too closely on this parallelism of lack of lipase and lack of toleration for fat; however, the experimental fact fits in so closely with clinical experience that one cannot refrain from suggesting the correlation. It may well be that this fat intolerance is based on more complex functional disturbances. With this same qualification it should be remembered that a study of the metabolism of alimentary intoxication[32] has shown that in this condition, even after the acute period, there is poor absorption of fat and an increase of neutral fat in the stools.

Conclusions (Part II)

In Part 1, which considered the pancreatic secretions in chronic malnutrition in infants, it was found that the various ferments of the pan-

[32]Meyer, Ludwig F.: Jahrb. f. Kinderh., 1907, LXV, 585.

creas are normally secreted even in advanced instances of marasmus or atrophy. In the present study of an *acute* disease, of acute intestinal indigestion, or alimentary intoxication, which was carried out also by the direct method, by the use of the duodenal catheter, the lipase was found deficient, although the two other pancreatic ferments were present in considerable amount. The deficiency of lipase seemed to be to some degree characteristic of this disturbance; it is not a general characteristic of all febrile conditions, and was not met with in pneumonia or empyema. It is possible that the lack of lipolytic activity in this disease should be correlated with the clinical manifestation of fat intolerance, and the metabolic studies showing a deficient absorption of fat.

CULTURAL AMEBÆ. A STUDY IN VARIATION

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Careful study of the life histories of various types of protozoa has fully demonstrated that fixity of type must be interpreted within wide limits. Variations in form and size may be due to stages in the life cycle or to varying conditions of the environment. Illustrations of life cycle changes are found abundantly in the group of rhizopods and foraminifera, and many cases, drawn from all groups, of the protozoa, might be cited to illustrate the effects of a changed environment on form and structure. Trypanosomes and other flagellates, infusoria and heliozoa have been particularly favorable subjects for the study of this phenomenon which has led to the axiom in protozoölogy that no species can be accurately defined until, not only its full life history but also its variations and modifications under various conditions of environment have been ascertained.

In no group of protozoa is the need of this study of environmental conditions more apparent than in that of the smaller amebæ, especially the parasitic and commensal forms. Here the specific differences are minute, and species, as at present recognized, shade one into another in so bewildering a fashion that taxonomy of the group is highly artificial. A glance at a table of the claimed differential features of the amebæ types found in the human intestine, for example that given by Craig[1] for *Entamœba histolytica, Entamœba tetragena,* and *Entamœba coli,* is sufficient to show how impossible it would be to place any ameba found in the human intestine, especially if the observer has sufficient knowledge of protozoa to understand their capacity to vary under different environmental conditions.

The failure to take into consideration the power of amebæ to vary widely has led to a number of loose statements, usually in the form of generalization from incomplete or inadequate observations and experiments. This is well illustrated by the attitude in some quarters toward cultural amebæ derived from the human intestine, the ground taken being that any ameba capable of cultivation on agar is ipso facto a free-living form derived possibly from cysts which have traversed the alimentary tract without change, or possibly from dust of the air. Thus Craig (1912)[2] states: "Later researches have shown that the amebæ which have been cultivated from dysenteric stools and the pus of liver abscesses are really free-living species, non-pathogenic in nature, and their presence in the stools and pus can be easily explained by communication of the material or the culture media by dust containing the cysts of the amebæ or their spores" (sic).

The truth is that much more minute work must be done (and can be done) in the study of the viable amebæ from the human intestine before the question of the relationship of "cultural amebæ" to "parasitic amebæ" can be considered settled.

Under the name of *Amoeba lobospinosa* Craig (1912) has described a viable ameba first cultivated from a dysenteric stool by Dr. Musgrave of Manila, and known by him as Ameba 11524. Some of this material was sent to the Army Medical Museum in 1905. In 1906, Dr. Musgrave was kind enough to send a culture labelled Ameba 11524 to one of us (Calkins) in New York. Presumably this is from the same ameba stock as that used by Dr. Craig. It has been under constant cultivation since 1907 by one of us (Williams) and forms the subject material of the experiments here described upon variations under different conditions of environment.

Comparatively few investigators have carried on extensive experiments with recorded observations on the minute morphological changes occurring in amebæ grown on artificial culture media. Of the few who have carried on such investigations, none has varied to any extent the conditions of growth. Thus Musgrave and Clegg (1904),[3] Vahlkampf 1905),[4] Noc (1909),[5] Nägler (1909),[6] Walker (1910),[7] Whitmore (1911)[8] and Craig (1912),[2] all grew their cultures at room temperature on the surface of slightly nutrient and more or less drying agar with certain bacteria as food. Some of these workers, it is true, tried other methods only to reject them in favor of this low-temperature surface-agar method. It is not strange, therefore, that they are practically all agreed as to the "characteristics" of cultural amebæ. In general terms they state that the resting trophozoite contains a single,, centrally situated nucleus, rarely two, of the "karyosome type," *i. e.*, "a relatively large and dense karyosome, containing all of the chromatin which is surrounded by an achomatic halo and bounded by a delicate nuclear membrane;" that it possesses with rare exceptions a contractile vacuole; that it reproduces by binary fission with mitotic division; and, finally, that it produces non-reproductive cysts each containing a single nucleus similar to that of the trophozoite. Because of these characteristics they claim that cultural amebæ are distince from those said to be parasitic in the intestinal tract of human beings, and that they belong to the genus Ameba (sic). Thus Walker (1911)[7] who agrees in the main with the others says that the type of amebæ found by him in cases of human amebiasis in Manila, "differs from the ameba type found in the Manila water supply and cultivated from the intestinal tract of man, in certain fundamental and biological characters. The trophozoite [of the former] has the nucleus situated excentrally instead of centrally in the resting organism; the chromatin is arranged peripherally instead of centrally in the nucleus; there is no contractile vacuole; reproduction by sporulation does not occur; and multiplication does not take place on ordinary artificial media. The cyst contains four or eight nuclei instead of a single nucleus; a reproductive process takes place in the cyst; and encystment occurs only in the body of the host." These characters, the author thinks, justify the establishment of the genus Entamoeba of Casagrandi and Barbagallo (Leidy).

Original work.—Ameba 11524 of Musgrave on which Craig worked and a strain of which was sent to us in 1906 as we have stated, is said

by Craig to be a "free-living" ameba entirely distinct from the "parasitic" forms.

Since 1911 this strain has been carried on by us in one series among others, in pure cultures (without other organisms) on brain-tissue medium. If the strain is grown at room temperature on the alkaline agar of Musgrave and Clegg with certain bacteria as food, the general morphological characteristics agree in the main with those as stated above. But when grown at high temperature (38° C.) on a very nutrient semi-solid medium (brain tissue), with added moisture (blood) from day to day, and with an easily assimilable bacterium that will not overgrow the medium at this high temperature (*e. g.,* one of the influenza group of bacilli), the difference in the morphology of these forms is remarkable. Some of the changes in structure have already been reported by one of us (Williams, 1911)[10] and this paper records a continuation of this study with particular reference to the changes in trophozoites and cysts produced by different temperatures and foods, amounts of moisture, and length of treatment. The variations noted among many have to do with (1) the number of nuclei, (2) the distribution of chromatin, (3) mitotic division, (4) the presence of a contractile vacuole, (5) and the structure of the cyst.

1. *The number of nuclei.*—As stated above, if the organisms are grown at room temperature on the usual culture medium employed by most investigators, there is normally only one nucleus in the trophozoite, although in exceptional cases there may be two; and practically all of the organisms encyst in from four to six days and remain encysted without further change until they are again placed on fresh agar when one ameba is liberated from each viable cyst. If, however, the organisms are grown in pure culture from trophozoites, on fresh sterile brain tissue as Williams[10] has reported, thus increasing both moisture and food, the organisms encyst much more slowly at room temperature, although they are not much changed otherwise. If, however, such a culture is placed at 34° C., the rate of division is decidedly increased and in three or four days a goodly number of trophozoites show two nuclei, while some show four or five. Such binucleated and multinucleated forms are slightly increased in numbers on the fifth and sixth days when encysted forms appear and gradually increase in number. If such a culture is placed at 38° C. the number of individuals containing multiple nuclei is still more increased, although not to a great extent (Photographs 3 and 5).

Now by adding to a similar series of cultures at different temperatures a bacillus which can be readily assimilated by the amebæ, the number of amebæ with multiple nuclei is greatly increased, especially at the highest temperature used (38°C.). While two and four nuclear forms (Photographs 1 and 6 and Figures 8 10, 16, and 17) are most frequent, individuals with eight nuclei are often found (Photograph 7 and Figures 11 and 22) and occasionally one with ten, eleven or twelve nuclei has been seen. There is a somewhat variable curve of increase in the number of multinucleated individuals according to the age of the inoculated amebæ and according to the position of growth, *i. e.,* at the middle of the brain

mass or at the edge. The following tables were made from a series which was inoculated simultaneously with a hemoglobinophile bacillus and a pathogenic yeast and observed and recorded for twelve successive days.

Even a casual examination of these tables shows the relative differences in number of nuclei of the trophozoites. At room temperature on ordinary culture media the trophozoites are described as uninucleated with "occasional" binucleated forms. In no case have percentages been given, so "occasional" has little value in comparisons, but we have found binucleated forms rarely in such cultures (less than .1 per cent). On brain-tissue media plus bacteria and yeast at 34° C. we find a slight difference in percentages of binucleated trophozoites in smears from the middle of the culture (Table I.) and from the edge (Table II.). Smears from the middle contained the highest number of trophozoites with two or more nuclei on the fifth day, these comprising eleven per cent. of the total number counted (forty-four). In smears from the edge of the culture, also on the fifth day, multi-nucleated trophozoites comprised 14.75 per cent. of all individuals counted.

On similar culture media at 38° C. there is a similar but reversed difference between smears from the middle and from the edge of the culture. The number of multinucleated trophozoites from the middle (Table III.) varies from nine and one-half per cent. (the lowest) on the twelfth day of culture, to thirty and one-fourth per cent. (the highest) on the fourth day. These are larger percentages than those found in smears from the edges of this culture (Table IV.). Here the numbers of multinucleated trophozoites vary from three and three-fourths per cent. on the ninth day, to twenty-eight and one-half on the second day.

The multinucleated forms may be somewhat unevenly scattered over the smear; in one field nearly every individual may contain more than one nucleus (Photograph 1), while in another nearly every one of the amebæ is uninucleated.

The multiple nuclei are formed by successive divisions, in the younger cultures apparently by mesomitosis (Photograph 2), the karyosome material helping to form the nuclear plate and with no pole bodies. Tripolar spindles have been seen occasionally but we have observed no trace of multiple division into eight nuclei as described for *Entamoeba coli* by Casagrandi and Barbagallo, and later by Schaudinn. In regard to this latter point, however, Hartmann (1912)[11] believes that these authors were wrong and that "parasitic amebæ" of man produce multinucleated forms in both trophozoites and cysts in exactly the same way that the present species (Am. 11524) on artificial culture media does.

In all of these cultures from the very beginning there may be an extrusion of fully formed, apparently perfectly normal nuclei, usually one at a time. Its significance and the conditions under which it occurs are still to be determined. That it is wholly a degenerative process as Darling (1913)[13] claims for *Entamoeba tetragena* growing in the intestinal contents of kittens, is doubtful since it occurs so frequently in young cultures.

TABLE I. 34° C.

Smears made from middle of culture

Days	Total Counted	Percentage Distribution of Nuclei in Trophozoites and Cysts in 1,900 Individuals from 2 to 6 Days in Culture												Remarks
		With 1 Nucleus		With 2 Nuclei		With 3 Nuclei		With 4 Nuclei		With 8 Nuclei		Dividing Forms		
		Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trohpozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	
2	400	92.25	.25	6.0	....	.75	....	....	....	....	....	.75	....	
3	400	94.0	.25	4.75	....	.75	....	....	....	....	....	.25	....	
4	400	91.0	....	7.75	....	.25	....	.75	....	.25	....	....	....	
5	400	87.25	.75	10.0	.50	.50	....	.50	.50	....	....	....	....	
6	300	91.33	3.0	4.0	1.32	.35	....	....	....	....	....	....	....	Only 300 counted because of yeast
7	...	....	....	...	....	....	....	....	....	....	....	....	....	Overgrown by yeast

TABLE II. 34° C.

SMEARS MADE FROM EDGE OF CULTURE

Days	Total Counted	Percentage Distribution of Nuclei in Trophozoites and Cysts in 3,600 Individuals from 2 to 11 Days in Culture														Remarks
		With 1 Nucleus		With 2 Nuclei		With 3 Nuclei		With 4 Nuclei		With 5 Nuclei		With 8 Nuclei		Dividing Forms		
		Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	
.....	400	89.50	0.50	7.0	....	0.25	....	0.50	....	....	....	....	....	2.25	....	
.....	400	85.00	5.50	8.75	....	0.50	....	....	....	0.25	....	....	....	....	....	
.....	400	88.75	5.00	6.0	....	....	....	....	.025	....	....	....	....	....	....	
.....	400	64.25	15.75	13.0	4.0	0.50	0.25	1.25	1.00	....	....	....	....	....	....	
.....	400	31.50	55.00	6.0	6.0	0.25	....	0.75	0.50	....	....	....	....	....	....	More or less overgrown by yeast.
.....	400	13.25	75.00	1.75	9.50	....	....	....	0.50	....	....	....	....	....	....	Many brown cysts. Only blue stained counted.
.....	400	8.00	8.00	2.0	9.0	....	....	0.25	0.75	....	....	....	....	....	....	Only blue stained cysts counted.
0.....	400	8.00	88.00	0.75	3.0	....	....	....	0.25	....	....	....	....	....	....	Only blue stained cysts counted.
1.....	400	0.0	99.75	....	0.25	....	....	....	....	....	....	....	....	....	....	All counted only 33 stained blue. No growth.

TABLE III. 38° C.

SMEARS MADE FROM MIDDLE OF CULTURE

Days	Total Counted	Percentage Distribution of Nuclei in Trophozoites and Cysts in 4,000 Individuals from 2 to 12 Days in Culture																				Remarks
		With 1 Nucleus		With 2 Nuclei		With 3 Nuclei		With 4 Nuclei		With 5 Nuclei		With 6 Nuclei		With 7 Nuclei		With 8 Nuclei		With 9 Nuclei		With 10 Nuclei		Dividing Forms		
		Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	
2.....	400	76.0	.25	19.50	....	1.25	....	1.50	....	....	....	....	....	....	....	.25	....	....	....	....	....	1.25	....	Many karyosomes in ring form
3.....	400	69.5	1.0	21.0	....	2.25	....	4.0	....	....	....	.5	....	....	....	.5	....	....	....	....	....	1.25	....	Peripheral chromatin rich. Nuclei excentric
4.....	400	66.25	1.75	23.5	1.5	1.75	....	4.0	.25	....	....	.25	....	....	....	.5	.25	....	....	....	....	....	....	
5.....	400	67.5	2.25	20.75	.5	2.0	....	4.0	.5	1.0	....	.5	....	.5	....	.25	....	....	....	....	....	.25	....	
6.....	400	66.25	2.75	20.75	1.75	1.75	....	4.75	.25	.25	....	.75	....	....	....	.25	.50	....	....	....	....	....	....	
7.....	400	66.25	1.50	23.75	....	2.50	....	3.25	.25	.50	....	.75	....	.50	....	.75	....	....	....	....	....	....	....	Karyosome becoming smaller
9.....	400	36.50	18.75	18.25	11.50	3.50	.50	3.75	4.75	.25	....	.75	.75	....	....	.25	.25	....	....	.25	....	....	....	Karyosome much smaller
10.....	400	27.50	30.50	19.00	12.75	1.50	.50	3.25	3.50	.50	....	.50	....	....	....	....	.25	....	....	.25	....	....	....	A chromatic network of "coli" type
11.....	400	40.25	29.00	13.00	10.25	.50	.25	2.50	2.75	.25	....	.50	....	....	....	.50	....	.25	....	....	....	....	....	Karyosome very small. Nuclei "histolytica" type
12.....	400	33.25	30.00	6.25	15.25	.25	.25	2.50	9.25	.25	....	....	2.25	....	....	.25	.25	....	....	....	....	....	....	Growth obtained after 4 days

TABLE IV. 38° C.

Smears Made from Edge of Culture

Days	Total Counted	Percentage Distribution of Nuclei in Trophozoites and Cysts in 4,000 Individuals Selected at Random on Smears 2 to 12 Days in Culture																Remarks
		With 1 Nucleus		With 2 Nuclei		With 3 Nuclei		With 4 Nuclei		With 5 Nuclei		With 6 Nuclei		With 8 Nuclei		With 10 Nuclei		Dividing Forms		
		Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	Trophozoites %	Cysts %	
2	400	70.25	0.25	23.75	....	2.50	....	2.00	....	0.25	....	....	....	....	....	....	....	1.00	....	
3	400	85.50	2.50	9.50	0.50	0.75	....	0.75	....	....	....	....	....	....	....	....	....	0.50	....	
4	400	49.00	17.00	14.50	8.50	2.25	0.50	3.50	3.00	0.25	....	0.75	0.25	0.25	....	....	0.25	....	....	
5	400	52.00	31.25	7.50	3.75	1.75	2.75	0.25	....	0.75	....	....	....	....	....	....	....	....	....	
6	400	35.50	24.75	16.75	11.00	0.75	0.25	3.25	4.00	0.25	....	1.25	1.50	0.50	0.25	....	....	....	....	
7	400	40.75	52.00	4.25	2.75	....	....	....	0.25	....	....	....	....	....	....	....	....	....	....	
9	400	24.00	65.50	3.25	5.00	0.25	....	....	1.75	....	....	....	....	0.25	....	....	....	....	....	Karyosome small
10	400	14.50	53.75	6.25	17.00	0.50	0.50	1.25	3.75	....	....	0.75	1.50	....	0.25	....	....	....	....	Staining inadequte to make out structure
11	400	30.00	37.50	8.25	15.50	0.50	0.75	2.00	5.00	....	....	0.25	0.25	....	....	....	....	....	....	
12	400	5.00	65.75	....	21.00	....	0.75	....	5.50	....	....	....	1.25	....	0.50	....	0.25	....	....	Growth obtained in 24 hours

2. *The distribution of chromatin.*—The moist cultures on brain media with bacteria, and at room temperature, show nuclei with comparatively little peripheral chromatin, although with more than in cultures grown on surface agar. When grown at the higher temperatures, the peripheral chromatin forms a wide band about the large karyosome, with an achromatic band between (Photograph 3, Figure 1). At this stage the karyosome is often in the form of a distinct ring with a central portion that stains feebly or not at all containing sometimes from one to several granules (Plate VI., Photograph 4 (a) and Plate III., Figures 2, 3, 4, and 5).

At about the sixth or seventh day of the culture the karysome becomes smaller, the peripheral chromatin more scattered over an achromatic network extending from the now distinct but delicate nuclear membrane to the karyosome (Plate III., Figures 6 and 7, and Plate VII., Photograph 10). These changes become more marked on the three to four following days (Plate III., Figure 8, and Plate VII., Photograph 11), the chromatin in the cysts showing in general the same changes as in the trophozoites (Plate IV., Figures 18 to 23 inclusive). Chromidia formation has not been observed although degenerated yeast cells and bacteria, in rounded amebæ having the appearance of the chromidia-containing "cysts" without a definite cyst wall as described by so many authors, have been seen frequently and they closely simulate the "vegetative chromidia" described as occurring in *Entamoeba tetragena* (Plate III., Figure 10).

3. *Mitotic division.*—In the early cultures up to the fourth day, definite mitotic division of the nucleus (Plate V., Photograph 2), and of the mesomitotic type, may be seen rather frequently (see Tables II. and III.). Occasionally an individual may be found with two and four spindles or with a tripolar spindle. After the fifth day definite mitosis has rerely been seen in the rather infrequent observations made on these cultures (spreads were made once a day about noon. Some evidence of promitosis has been found in individuals from the older cultures. Again the amebæ in these older moist cultures resemble the forms described as parasitic and pathogenic for man.

4. *The contractile vacuole.*—The ordinary trophozoites of this strain grown on the usual culture medium have each a single contractile vacuole, but this vacuole disappears in specimens from the culture mass after the fifth day. The organisms are motile and normal in other ways, the culture containing them is always viable when transplanted on fresh culture media and in twenty-four hours in these transplanted cultures many young forms, each showing a contractile vacuole, are present.

5. *Encysted forms.*—Grown on the surface of the usual agar medium the cysts are mononuclear, a fact claimed as characteristic of all the cysts of cultural amebæ. Grown at high temperature (34°–38°) on brain media without bacteria, the number of nuclei in the cysts increases, many having two and some four (Plate VI., Photograph 4 (b)). Grown with bacteria at these higher temperatures the cysts with two and four nuclei increase in numbers while individuals with six, eight, ten, and

twelve (Plate V., Photograph 3, and Plate IV., Figures 20 to 23 inclusive) nuclei are found and an occasional one is seen showing an odd number of nuclei up to eleven (Plate IV., Figure 19). Only a single spindle, however, has been infrequently observed in a cyst, the majority of the multinucleated cysts seem rather to be formed after division of the nucleus has taken place in the pre-cystic stage. In the cultures there are many rounded forms of amebæ similar to those pictured as the first definite stages of cyst formation by practically all investigators, and these frequently show mitoses, but when the cyst wall is present such mitoses are rare.

The minute study of the further history of the multinucleated cysts is still to be made. We do know, however, that multinucleated individuals within cysts may leave their cysts still as multinucleated individuals when transplanted upon fresh culture media, and that these then may divide into daughter forms equalling in number the number of nuclei present in the encysted individual.

Occasionally, within a cyst a binucleated form has been found divided into two complete individuals but not more. The great majority of multinucleated forms, so far as observed, remain multinucleated in the cyst until they degenerate, unless transplanted. No evidence of conjugation or autogamy has been found. In rounded or oval forms without definite cyst walls the presence of a central vacuole with a nucleus on either side or with one or more nuclei irregularly placed is often seen. It frequently occurs in individuals having from one to six nuclei (Plate IV., 12 to 17, and Plate VII., 8 and 9). So far as this characteristic also is concerned, therefore, these cultural forms are parallel with the "parasitic" forms recently described by Hartmann.

Summary of Conclusions

The variations shown by cultural amebæ from the intestine when subject to experimental control are sufficient to indicate that forms described as parasitic in the human intestinal tract, may likewise vary under differing conditions. We have no reason whatsoever to doubt that No. 11524 is capable of living in the human digestive tract, nor that it may there vary in one or in all of the ways observed when grown on culture media. These variations, furthermore, were it an intestinal form, might lead to its confusion with those forms described as pathogenic. The type of nucleus, for example, varies from that of *Vahlkampf's limax,* through *E. tetragena* type to the type of *E. histolytica.* If specimens of this strain (Am. 11524) were found in feces, with a large karyosome and with but little peripheral chromatin or none at all, they might be described as "free-living" forms belonging to the genus Ameba. If other characteristic specimens from the same strain were found with a broad zone of chromatin forming the periphery of the nucleus and enclosing a karyosome often in the form of a ring with or without one to several granules, within it, they might be described as *Entamœba tetragena* (Plate VI., Photograph 4 (a) and Plate III., Figures 2, 3, 5, and 7). If still other characteristic specimens were found with small karyosomes

and peripheral chromatin extending through an achromatic network from the karyosome to the nuclear membrane, they might be described as *Entamoeba coli* or as *E. histolytica.*

(We might call attention in this connection to the fact that in the earlier studies on this culture and others isolated from the intestines of mammals, one of us (Williams) made a few feeding experiments with kittens and puppies. From the stools of these animals so few amebæ were found in hanging drops of smears that nothing could be judged from their morphology. Only mononuclear organisms were seen showing nuclei with medium sized karyosomes and a moderate amount of peripheral chromatin. Evidently the amebæ had been overgrown by the intestinal flora of that particular time, as cultures were recovered from the stools with difficulty. Of course, studies on animal inoculations with cultures, repeated on a much larger scale with sufficient controls, give promise of solving many of the questions related to the vexing subject of species in this group of amebæ.)

Nor do the cysts escape the variations. Much apparently depends upon them for the indentification of "parasitic" from "cultural" amebæ, the cultural forms "always" having one nucleus. Table V., however, shows that at 34° C. as many as nine and one-half per cent. of the total number of these "cultural" organisms may have two nuclei and one per cent. may have four nuclei in the cysts, while at 38° C. twenty-one per cent. of all of the amebæ may have two nuclei and more than nine per cent. may have four nuclei in the cysts. If some of these tetranucleated cysts were found in feces they might well be described as cysts of E. tetragena. Under some conditions, also, cysts with eight nuclei form one-half of one per cent. of all the organisms. If such cysts were observed alone, they might well be described as E. coli. Table V. shows the relations of these multinucleated cysts to the uninucleated cysts.

Similarly with regard to the presence of the contractile vacuole. It is well known that fresh water heliozoa if transferred to salt water by slow stages will lose the contractile vacuole and regain it when brought back to fresh water. So this ameba under different experimental conditions will lose and regain its contractile vacuole but will still be the same ameba.

We do not wish, however, to lay too much stress upon these experimental results; conditions of the cultures might be such as to bring about variations when these do not occur under conditions "normal" to the organism. But it is not impossible that analogous variations occur with the forms described as pathogenic, and it is unwise for any one at present to be too positive in regard to the distinctive features of *Entamoeba coli, E. tetragena,* and *E. histolytica,* or any of the Entameba group. These may be in man, three or more, or two (as Hartmann, Whitman, Walker, Darling, and even Craig[15] now think) or possibly only one species of amebæ manifesting different forms under different conditions.

TABLE V

PERCENTAGES OF CYSTS TO TOTAL NUMBERS OF INDIVIDUALS COUNTED

Days	Middle of Culture at 34° C.			Edge of Culture at 34° C.			Middle of Culture at 38° C.			Edge of Culture at 38° C.		
	With 1 Nucleus	With 2 Nuclei	With 3 Nuclei	With 1 Nucleus	With 2 Nuclei	With 3 Nuclei	With 1 Nucleus	With 2 Nuclei	With 3 Nuclei	With 1 Nucleus	With 2 Nuclei	With 3 Nuclei
2	.25			.50			.25			.25		
3	.25			5.50			1.00			2.50	.50	
4				5.00		.25	1.75	1.50	.50	17.00	8.50	4.00
5	.75	.50	.50	15.75	4.00	1.25	2.25	.50	.50	31.25	3.75	2.75
6	3.00	1.32		55.00	6.00	.50	2.75	.75	.75	24.75	11.00	6.00
7				75.00	9.50	50	1.50		.25	52.00	2.75	.25
9				80.00	9.00	.75	18.75	11.50	5.75	65.60	5.00	1.75
10				88.00	3.00	.25	30.50	12.75	3.75	53.75	17.00	6.00
11				99.75	.25		29.00	10.25	2.75	37.50	15.50	6.00
12							30.00	15.25	11.75	65.75	21.00	7.25

From this table, especially from the last series, it will be seen that the number of cysts containing two or more nuclei is more than half as large as the number containing one nucleus. Thus on the 4th day, from the edge of the culture at 38° C. 12½% of all individuals were multinucleated cysts, while 17% were uninucleated. On the 6th day the relation was 17% to 24.75%. On the 11th day it was 21½% to 37½%.

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Description of Figures (Plates III. and IV.)

(Fixed in sublimate-acetic solution for five minutes. Stained with Delafield's hematoxylin. x 2,000. Grown on brain-tissue medium with a hemoglobinophilic bacillus.)

Plate III., Fig. 1.—Three-day trophozoite showing nucleus with large karyosome and wide band of peripheral chromatin.

Figs. 2–5 and 7.—Show various ring-formed karyosomes.

Fig. 2.—Double ring. Three-day culture.

Fig. 3.—Ring containing four small granules. Four-day culture.

Fig. 4.—Ring karyosome apparently alone—the peripheral chromatin of the nucleus masked by an ingested tetracoccus that had contaminated the culture. Four-day culture.

Figs. 5 and 7.—"Centriole" surrounded by a ring of chromatin from which more or less of a "linin" network extends to the peripheral chromatin of the nucleus. Six-day culture. 7, came from culture inoculated also with a pathogenic yeast which the amebæ readily ingested. Partly digested yeast-cells can be seen in one of the food vacuoles.

Fig. 6.—Six-day culture, showing smaller karysome.

Fig. 8.—Nine-day culture showing individual containing two nuclei each, reticular chromatin and much smaller karyosome.

Fig. 9.—Seven-day culture with "coli type" of nucleus and with nine red blood cells some partly digested. Fresh rabbit blood had been added to the culture twenty-four hours earlier.

Fig. 10.—Four nucleated trophozoite from a four-day culture. The four-nucleated individuals from older cultures contain smaller nuclei with smaller karyosomes.

F.G. 11.—Eight-nucleated trophozoite. Older cultures contain smaller nuclei.

Plate IV, Figs. 12 to 16.—Forms with large vacuoles and nuclei variously disposed.

Fig. 12.—Simulates an atypical division form. Four-day culture.

Fig. 13.—Simulates the breaking up into chromidia in Schaudinn's autogamy of "E. coli." Probably a degenerating form. Five-day culture.

Fig. 14.—Like one of the stages of Schaudinn's autogamy in "E. coli." Five-day culture.

Fig. 17.—Like the "reduction" stage in Schaudinn's E. coli. Eight-day culture.

Figs. 18-23.—Multiple nuclei in cysts with definite cyst walls which are not much contracted in moist cultures.

Figs. 18–20.—Five-day culture, showing larger nuclei and karyosome.

Figs. 21–23.—Nine-day culture, showing smaller nuclei and karysomes

Descriptions of Photographs.[1] (Plates V.-VII.)

All spreads fixed in sublimate-acetic solution for five minutes. Numbers 1, 6, 8, 11 stained with iron hematoxylin. No. 1: x 100, No. 3: x 1,000, the others x 2,000.

Plate V, Photograph 1.—Large number of multinucleated amebæ in one field. Peripheral chromatin of nuclei not well brought out. Four-day brain-tissue culture with bacteria at 38° C.

Photograph 2.—Mitotic division. Three-day pure culture on brain-tissue medium at 34° C.

Photograph 3.—Large forms containing two fully developed nuclei each with large karyosome and wide band of peripheral chromatin. Three-day pure culture on brain-tissue medium at 38° C.

Plate VI, Photograph 4.—Four-day pure culture on brain-tissue medium at 38° C. (a) Ring form of karyosome showing "centriole." (b) Two nuclei in beginning cyst.

Photograph 5.—Four nuclei in trophozoite. Three-day pure culture on brain-tissue medium at 38° C.

[1]Photographs not well reproduced in these prints.

PHOTOGRAPH 6.—Four nuclei in trophozoite with peripheral chromatin fainter than in No. 5. Four-day pure culture on brain-tissue medium with hemoglobinophilic bacilli.

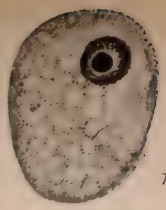
PHOTOGRAPH 7.—Same culture, five-days. Form showing eight nuclei (one not in focus) a binucleated form with smaller karyosomes at side.

PLATE VII, PHOTOGRAPHS 8 and 9.—Vacuolated forms. 8, four nuclei (one not in focus) at one side of large vacuole. 9, four nuclei arranged around a large vacuole (one nucleus faintly seen through vacuole). Five-day brain-tissue culture with influenza bacillus.

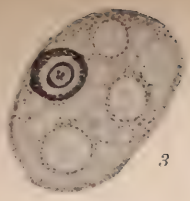
PHOTOGRAPH 10.—Individual with two nuclei of the reticular type with smaller karyosome. Seven-day brain-tissue culture with influenza bacillus.

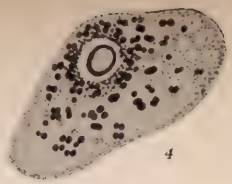
PHOTOGRAPH 11.—Individual with two nuclei of the reticular type with still smaller karyosome. Nine-day brain tissue culture with influenza bacillus.

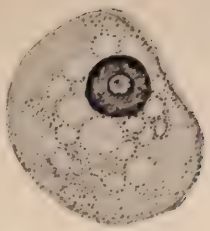
PHOTOGRAPH 12.—Beginning cyst (shown by deeper stain and no vacuoles) with four nuclei (one not in focus). Six-day brain-tissue culture with influenza bacillus at 38° C.


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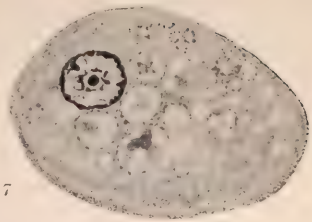

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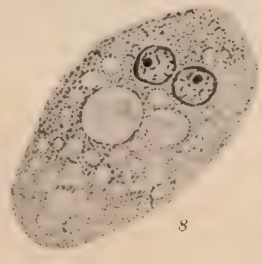

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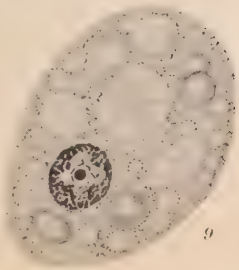

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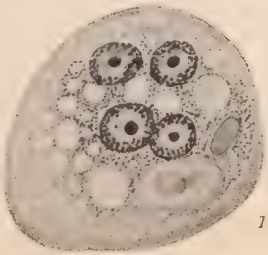

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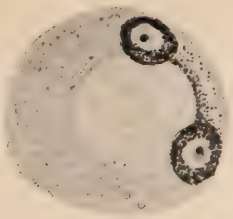

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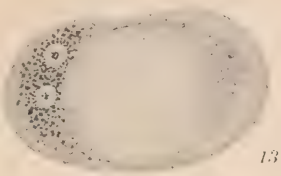

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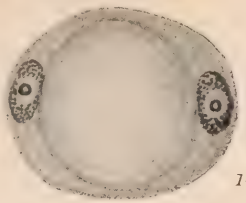

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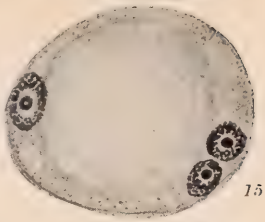

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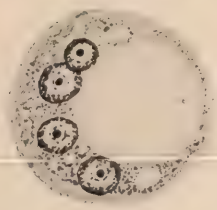

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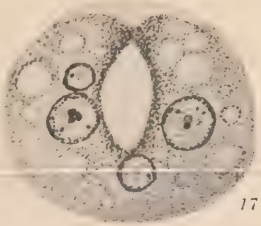

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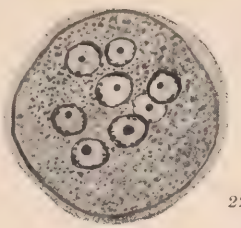

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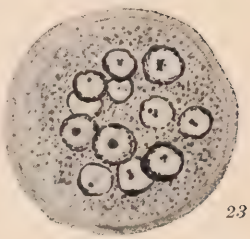
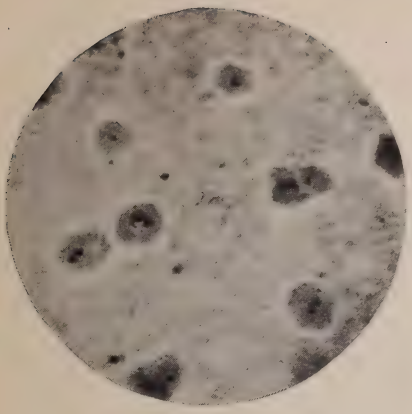
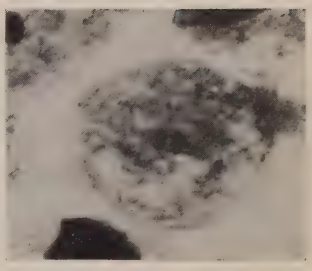

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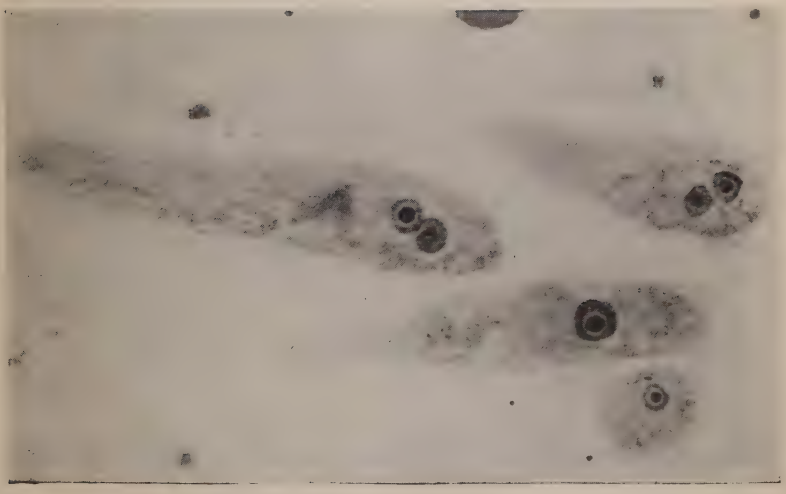
PLATE V



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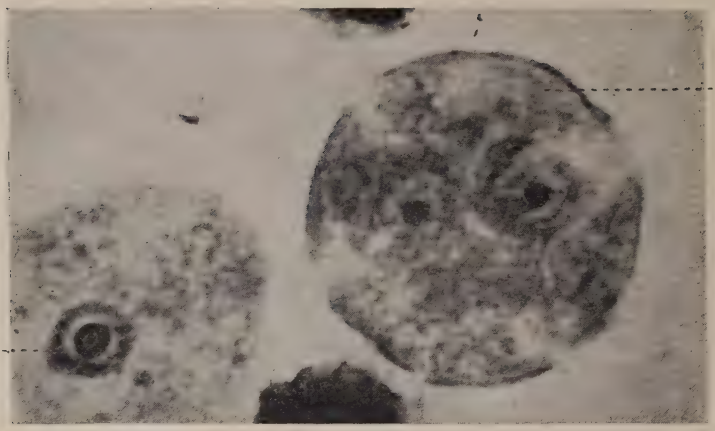


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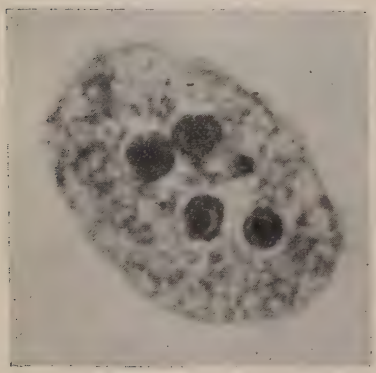


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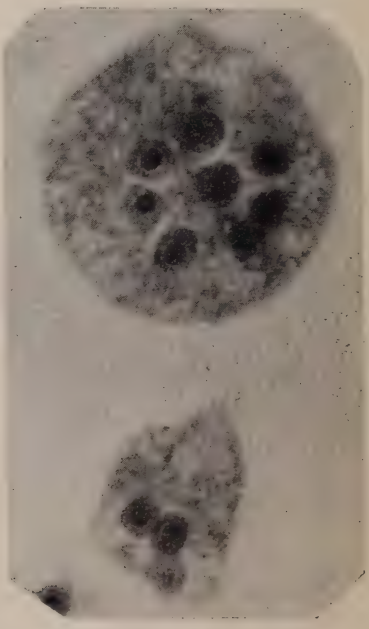
PLATE VI



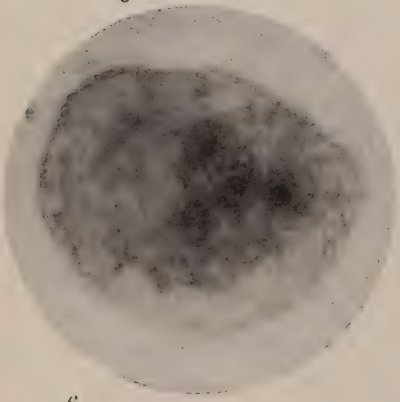
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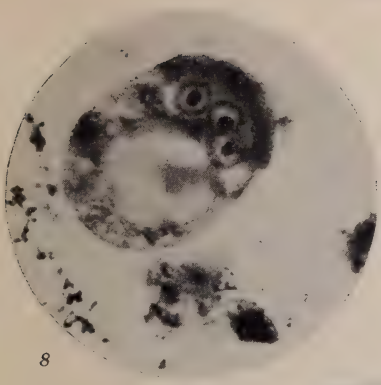


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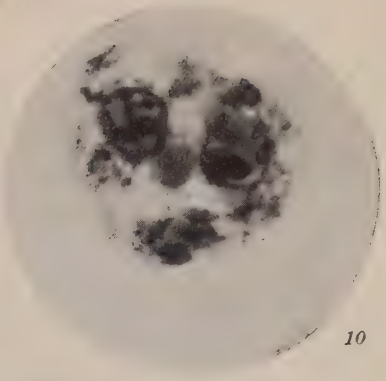


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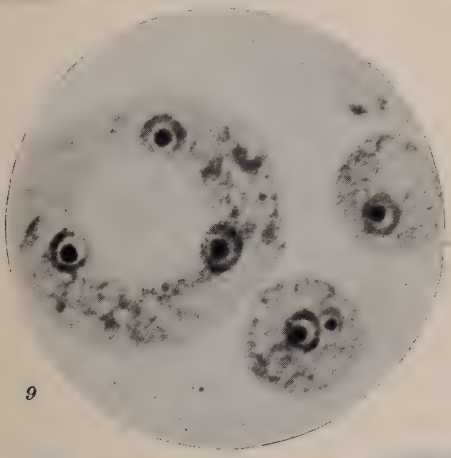
PLATE VII



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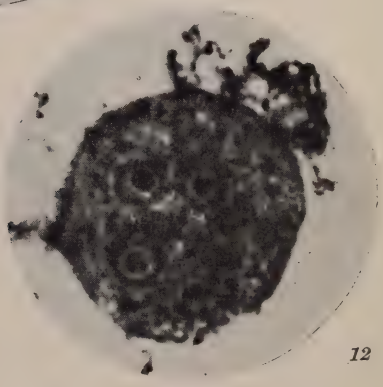
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